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AMPHENONE: TOXICITY AND EFFECTS ON ADRENAL AND THYROID FUNCTION IN MAN

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AMPHENONE[1] has been found to alter thyroid and adrenal function in the rat and dog. In addition, this compound exhibits a weak progestational action on the rabbit uterus and an atypical folliculoid effect upon the genital tract of the rat (1). Of these multiple endocrinologic effects the most pertinent to the present report is the capacity of amphenone to cause a marked depression of adrenocortical secretion in the dog. This is manifested by a pronounced reduction in the corticosteroid content of the adrenal venous effluent in the hypophysectomized dog, even in the presence of high levels of exogenous corticotropin (2, 3).

Studies of the possible effects of amphenone in man were undertaken in view of the growing clinical interest in the control of endocrine function in patients with breast cancer (4). Accordingly, the response to amphenone has been observed in 24 patients with cancer, adrenal hyperplasia, or thyrotoxicosis. Special emphasis was laid upon signs of toxicity and alterations in adrenal and thyroid function.

MATERIALS AND METHODS

The patients studied were hospitalized throughout the investigation on a research ward. Diet and activity were not limited except as noted in the case abstracts. Quantita-

[1] Amphenone: 1,2-bis(*p*-aminophenyl)2-methyl propanone-1 dihydrochloride, originally synthesized by Allen and Corwin, *J. Am. Chem. Soc.* **72**: 117, 1950.

tive values were determined for a comprehensive series of clinical indices before, during, and after amphenone administration. These included the following:

I. Adrenal Function
   A. Urinary excretion of 17-hydroxycorticoids by the method of Silber and Porter (7); normal range, 2–10 mg. per twenty-four hours.
   B. Urinary excretion of 17-ketosteroids by the method of Callow *et al.* (8); normal adult range, 4–18 mg. per twenty-four hours.
   C. Plasma 17-hydroxycorticosteroid concentration by the method of Silber and Porter (7) as modified by Peterson *et al.* (9); normal range 5–25 micrograms per 100 ml.
   D. Response to exogenous corticotropin administered either in doses of 100 units of gel intramuscularly or in doses of 40 units given intravenously during either a 4-hour or an 8-hour period.
   E. Eosinophil counts.
   F. Frequent blood pressure determinations.
   G. Serum electrolyte determinations.

II. Thyroid Function
   A. Thyroidal I^{131} uptake in twenty-four hours following a dose of approximately 10 microcuries by mouth; normal range, 15–45 per cent.
   B. Serum protein-bound iodine (PBI) concentration by the method of Zak *et al.* (10); normal range, 3.5 to 8 micrograms per 100 ml.
   C. Basal metabolic rate (BMR).
   D. Serum cholesterol level.
   E. Blood I^{131} concentration; all samples were counted in identical thin glass containers in a well-type scintillation counter.

III. General Metabolic and Clinical Status
   A. Complete blood count.
   B. Urine analysis.
   C. Fasting blood sugar level and glucose tolerance tests.
   D. Cephalin flocculation (blood).
   E. Thymol turbidity (blood).
   F. Serum bilirubin concentration.
   G. Total plasma protein level and albumin/globulin ratio.
   H. Bromsulphonphthalein retention in forty-five minutes (blood).

Continuous urine collections (in 24-hour specimens, promptly refrigerated) were maintained throughout the studies.

Constant clinical surveillance was maintained in order to evaluate any evidence of toxicity as manifested by undue drowsiness, gastro-intestinal symptoms, skin eruption, discoloration of skin and mucous membranes, or hepatic enlargement. Drugs which depress the central nervous system, especially narcotics, were discontinued whenever clinically feasible before starting amphenone, in order to obviate potential additive effects. Amphenone was administered orally in capsules containing 0.125 to 0.5 gram. Dosage intervals varied between two and six hours.

Photographs and x-ray films were made at appropriate intervals in order to detect any possible effect upon the primary tumor or metastases.

CASE REPORTS

Table 1 summarizes the effects of amphenone in our series. Abstracts of 9 of the more completely studied cases follow:

Case 1

M.C. was a 27-year-old woman with carcinoma of the breast and extensive osseous and soft-tissue metastases. She had had radical mastectomy, ovariectomy, and x-ray therapy to the metastatic lesions. Amphenone was given for thirty-six days in dosages of 0.5 to 6.0 grams a day at a rate of 0.5 gram every two to four hours. She experienced some increase in her previously slight nausea and vomiting and became drowsy. On the seventeenth day of treatment, cyanosis without dyspnea was noted and the presence of methemoglobinemia was confirmed spectrophotometrically. Liver function tests yielded completely normal results on the eighth and twenty-seventh days of treatment. However, on the thirty-fourth day cephalin flocculation was 3 plus in forty-eight hours and the serum bilirubin level was 8.1 mg. per 100 ml. Oliguria (110–350 ml. per day) appeared on the thirtieth day of treatment, despite adequate fluid intake. Control serum electrolyte and blood urea nitrogen levels had been normal, but on the thirty-fourth day of treatment the concentration of serum sodium was 111 mEq./L, potassium 5.5 mEq./L, and blood urea nitrogen 23 mg. per 100 ml., and there was marked edema over the sacrum. She was given 300 ml. of 5 per cent physiologic saline intravenously and the serum sodium level rose to 137 mEq./L, but she expired on the thirty-sixth day of treatment The blood pressure had been low (around 100/50) during the episode of hyponatremia. During the last six days of life, the dosage of amphenone was between 0.5 and 2.0 grams a day.

The urinary 17-ketosteroids remained normal and the urinary corticosteroids slightly low (0.6–2.0 mg. per day) before and during treatment. The effect of intramuscular corticotropin on urinary corticosteroid excretion was somewhat impaired during amphenone therapy, the control rise being from 1.2 to 12.3 mg. per day and that during treatment from 2.7 to 6.2 mg. per day. The thyroidal radioiodine uptake fell from a high control value of 61 per cent to 5 per cent on the eleventh day of treatment, but corresponding BMR determinations were −8 and +7 per cent, respectively.

Postmortem examination showed widespread metastatic disease. The liver was grossly enlarged, weighing 1,650 Gm. It was estimated that no more than 1 per cent of the liver was displaced by tumor tissue. There were approximately 10 tumor nodules, each 0.5 to 1.5 cm. in greatest diameter in the dome of the liver.

There were several large tumor-laden lymph nodes around the porta hepatis, but the common bile duct was entirely normal and these nodes did not appear to obstruct the duct. Histologically, there was evidence of degeneration of many hepatic cells without inflammatory infiltrate, most severe near the central veins. There were numerous lymphocytes and a few polymorphonuclear leukocytes in the portal connective tissue. No marked changes were seen in the kidneys, or urinary tract. The thyroid was small and most of the follicles were small and contained no colloid. The epithelial cells were tall-columnar but there was no papillary infolding or lymphocytic infiltration. The pituitary appeared normal. The right adrenal gland measured 6×7×1 cm. and weighed 15.4 Gm. The cortex averaged 2 mm. in width, the most peripheral zone being yellow but the remainder being a golden-brown. The left adrenal was abnormal in shape and size, measuring 9.5×3×2.5 cm. and weighing 19 Gm.; the inferior pole fused with metastatic carcinoma. There were several 2–3 mm. nodules of metastatic tumor in each adrenal, lying principally in the medulla. Microscopically all sections showed hyperplasia of the cortical cells in all three zones, most marked in the fasciculata. There were numerous foci of large vacuolated cells in the cortex. These contained large amounts of sudanophilic and doubly refractile material.

TABLE 1. EFFECTS OF AMPHENONE

Patient				Diagnosis	Amphenone: dose & duration	Effect on adrenals	Effect on thyroid	Toxic and other effects
No.	Inits.	Age	Sex					
1	M.C.	27	F	Ca. of breast	0.5 to 6 Gm./day for 36 days	Yes (*see* text). Adrenals enlarged at autopsy	Yes	Drowsiness, methemoglobinemia, hyponatremia, impaired liver function.
2	E.C.	73	F	Ca. of breast	2 to 7 Gm./day for 53 days	No	Yes	Drowsiness, bad dreams, ? anemia.
3	G.D.	71	F	Ca. of breast	1 to 2 Gm./day for 34 days	Yes	Yes	Drowsiness, rash, hyponatremia, heartburn, nausea, vomiting, methemoglobinemia.
4	I.C.	47	F	Ca. of breast	500 mg./day for 25 days; 1.5 to 3 Gm./day for 30 days; 4 Gm./day for 10 days	—* Yes Yes	Yes Yes Yes	Drowsiness, heartburn, anorexia, nausea, vomiting, methemoglobinemia, hepatomegaly.
5	C.B.	24	M	Thyrotoxicosis	2 to 3 Gm./day for 21 days	No	No	Slight heartburn. Doses too small and too infrequent.
6	J.T.	25	F	Thyrotoxicosis	3 to 8 Gm./day for 21 days	Yes	Yes	Nausea, vomiting, rash, methemoglobinemia.
7	R.H.	49	F	Exophthalmos	4 Gm./day for 10 days	Yes	—	Heartburn.
8	N.G.	35	F	Adrenal ca.	1 to 12 Gm./day for 34 days	Yes	Yes	Drowsiness, nausea, vomiting, methemoglobinemia.
9	O.S.	29	M	Adrenal hyperpl. & adenoma	2.5 to 4 Gm./day for 15 days	Yes	Yes	No toxic symptoms.
10	S.S.	15	F	Adrenogen. syn.	3 Gm./day for 5 days	No†	—	Heartburn.
11	R.E.	41	F	Ca. of breast	1 to 3 Gm./day for 5 days	No†	—	Drowsiness, dizziness.
12	A.M.	39	F	Ca. of breast	1 to 3 Gm./day for 4 days	—	—	Drowsiness, bad dreams.
13	L.R.	29	F	Ca. of cervix	2 to 12 Gm./day for 24 days; 6 Gm./day for 11 days. Stopped 2 days before death	Adrenals enlarged at autopsy; rt. 16 Gm., lt, 19 Gm.	—	Drowsiness, methemoglobinemia, impaired liver function, rash; bilat. urethral obstruction by tumor at autopsy.
14	L.I.	41	F	Ca. of cervix	6 to 8 Gm./day for 7 days; same later for 12 days	—	—	Drowsiness, methemoglobinemia (not proved spectroscopically) eosinopenia.
15	W.C.	55	M	Ca. of prostate	3 Gm./day for 5 days; 6 Gm./day for 22 days	No†	—	Drowsiness, bad dreams.

* Not studied.

† Only 17-ketosteroids measured.

TABLE 1. (*continued*)

Patient				Diagnosis	Amphenone: dose & duration	Effect on adrenals	Effect on thyroid	Toxic and other effects
No.	Inits.	Age	S x					
16	L.I.	29	F	Ca. of cervix	1 to 6 Gm./day for 20 days; same later for 15 days	No†	—	Drowsiness, rash; died of metastases during second course of amphenone.
17	L.S.	60	M	Ca. of prostate	3 to 8 Gm./day for 8 days	—	—	Drowsiness, weakness, nausea, hypotension.
18	D.S.	54	F	Ca. of breast	3.5 Gm./day for 10 10 days	—	—	Drowsiness, epigastric discomfort, anorexia.
19	S.M.	48	F	Ca. of breast	2 to 3 Gm./day for 2 days; 3 to 7 Gm./day for 3 days; 6 Gm./day for 2 days; 2–3 Gm./day for 15 days	No†	—	Nausea, vomiting, lethargy, rash.
20	R.A.	56	F	Ca. of breast	3 Gm./day for 3 days	No change 17-ketos. or blood cort.	—	Nausea, vomiting.
21	F.S.A.	46	F	Mult. myeloma	1.5 to 4 Gm./day for 7 days	—	—	Drowsiness, vivid dreams, rash.
22	M.P.	37	F	Ca. of breast	2.5 to 4 Gm./day for 14 days	No change urinary 17-ketos. or corticoids or blood corticoids	—	Drowsiness; fewer narcotics, but carcinoma progressed during amphenone treatment.
23	E.M.	70	F	Ca. of breast	1 to 3 Gm./day for 19 days	No change in 17-ketos.	—	Extreme drowsiness.
24	C.L.	28	F	Ca. of breast	1.75 to Gm./day for 4 days	No change in 17-ketos. or urinary corticoids	—	Drowsiness, bad dreams.

Case 2

E.C. was a 73-year-old woman who had had a radical mastectomy for carcinoma of the breast in 1926. She had several x-ray treatments after the operation, then had no further treatment or sign of recurrence until 1945, when she received irradiation to the left shoulder region for adenocarcinoma thought to be metastatic from the initial lesion. The carcinoma progressed despite further irradiation and despite several courses of androgens and estrogens.

In 1954 she exhibited extensive local soft-tissue metastases and a lytic lesion in one rib. She was given amphenone for fifty-three days in a dosage of 2.0 to 7.25 grams a day at intervals of three or four hours.

There was no evidence of alteration of the urinary 17-ketosteroid or corticosteroid levels during treatment, and the eosinophil counts throughout showed no change from controls. While the patient was receiving the highest dosage of amphenone, there was a

marked response of the blood corticosteroid level to administration of corticotropin (16 μg. before, and 96.6 μg. per 100 ml. after, intravenous injection of 40 units of ACTH over four hours). The condition of the patient at that time was such that accurate urine collections could not be made.

There was marked suppression of thyroidal function. The thyroidal radioiodine uptake fell from 42.5 per cent (control) to 9.5 per cent on the sixteenth day of treatment and remained at that level throughout treatment. The sixth day after stopping amphenone it was 75 per cent, the twelfth day 89 per cent, and the nineteenth day 49 per cent. On the fifty-first day of treatment, the serum PBI level was 0.5 μg. per 100 ml. and this value rose to 4.5 μg. per 100 ml. by the eighteenth day after treatment. There was no significant change in the cholesterol level or the BMR, both of which remained normal. The patient became extremely drowsy and confused when receiving the higher dosages of amphenone. There was no significant change in the cutaneous nodules of tumor during treatment.

Case 3

G.D. was a 71-year-old woman with carcinoma of the breast metastatic to the skull and to the left cerebellopontine angle, lumbar and dorsal spine, and pelvis. She was given 1–2 grams of amphenone daily for thirty-four days (except for one day on which she received only 0.75 gram because of her debilitatated condition) in doses of 0.25 or 0.5 gram every six hours. There was a slight fall in urinary corticosteroid excretion and a slight and irregular suppression of the response to corticotropin administered weekly as ACTH-gel (Fig. 1). Not only was there repeatedly a lower peak level after corticotropin, but the maximal response appeared twenty-four hours later than it did during the two control periods. The large response to corticotropin near the end of the treatment period

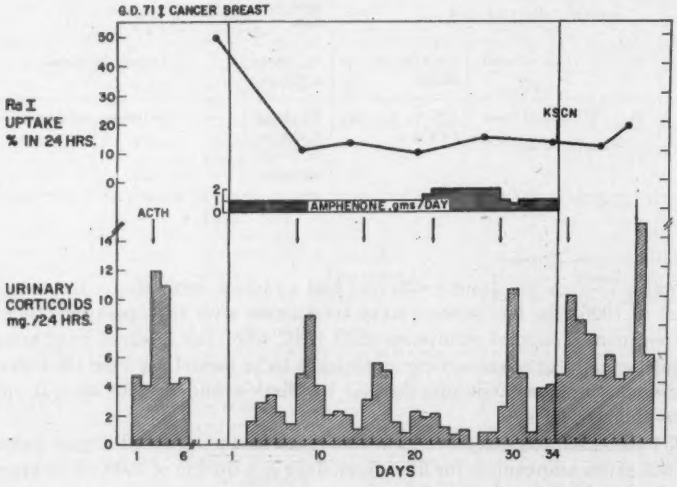

FIG. 1. G.D. (Case 3). Effect of amphenone on thyroidal radioiodine uptake, urinary 17-hydroxycorticosteroid excretion, and the response of the latter to injections of corticotropin (ACTH).

occurred on a day during which she received only 0.75 gram of amphenone. On the twenty-third day of treatment the patient appeared drowsy, and this progressed to an extreme degree on the twenty-eighth day of treatment (after which the dosage of amphenone was reduced and she improved slightly). On the same day the patient became oliguric (output 240 ml.) and the next day the blood urea nitrogen level was 61 mg. per 100 ml., although both the BUN and electrolyte concentrations had been normal during the control period. The following day after hydration, the serum electrolyte levels were: sodium 121, potassium 6.4, carbon dioxide 14, and chloride 105 mEq./L, and BUN 38 mg. per 100 ml. Methemoglobinemia was also demonstrated at this time. On the thirtieth, thirty-first and thirty-second days of amphenone therapy the patient was given 2 liters of 5 per cent dextrose and water, 500 ml. of physiologic saline, and 40 ml. of molar lactate daily by vein. However, the urinary sodium level rose from values of 4 and 7 mEq. per day just prior to this episode, to 60 mEq. per day but the concentration of serum sodium and other electrolytes remained unchanged, despite a fall in the BUN to 11 mg. per 100 ml. The day after stopping amphenone she was given 8 grams of salt daily by mouth; the serum sodium rose from 119 mEq./L on that day, to 138 mEq./L the following day.

The thyroidal radioiodine uptake was markedly depressed as shown in Figure 1. It was felt that the absence of a rebound to high levels after stopping amphenone was due to the administration of 2 grams of KSCN by mouth at the end of treatment in an effort to characterize the nature of the effect of amphenone on thyroid function. The BMR and serum PBI level remained normal during treatment.

On the eleventh day of treatment with amphenone, a morbilliform pruritic rash appeared on the patient's mid-back, then faded the following day with no specific treatment. She had been receiving chloral hydrate in the evenings and had had an injection of ACTH-gel three days before, but these medications were later continued without reappearance of the rash.

Case 4

I.C. was a 47-year-old woman with breast carcinoma metastatic to the pelvis, spine, skull, right temporal lobe, and regional lymph nodes. She had suffered a relapse after cranial decompression, again after a remission with methyltestosterone, and subsequently after x-ray therapy to the cranium.

She was given a 26-day course of amphenone in small dosages to find out whether these amounts had any significant thyroidal effect—receiving only 0.5 gram per day as 125 mg. every six hours. The control thyroidal radioiodine uptake was 26 per cent; this fell to 16 per cent during treatment (ninth day), but rose again while treatment was continued to 27 per cent. There was no change in the serum PBI level from the normal range during treatment. The thyroidal uptake the day after stopping treatment was 46 per cent.

After a control period of two weeks, the patient was given 1.5 to 3 grams of amphenone daily for thirty days at the rate of 0.5 Gm. every four hours. Corticotropin was given in a dosage of 100 units of ACTH-gel intramuscularly approximately once weekly. There was slight suppression of the basal urinary corticosteroid level (Fig. 2), and there was definite interference with the response to corticotropin during treatment. Also of note is the temporary rise in corticosteroid excretion to slightly higher than control levels when amphenone was stopped. The urinary 17-ketosteroids and their response to corticotropin were unchanged. The thyroidal radioactive iodine uptake fell from 46 per cent to 5.8 per cent within four days and remained at that level until thyroid-stimulating hormone was given for four days (10 units daily intramuscularly), when it rose to 34.5

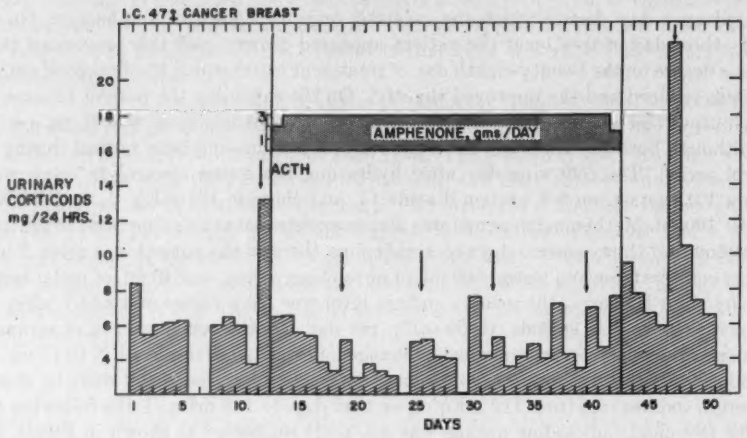

FIG. 2. I.C. (Case 4). Urinary 17-hydroxycorticosteroid excretion while receiving 3 grams of amphenone daily. There was definite interference with the response to corticotropin (ACTH).

per cent. During treatment, the liver was found to be enlarged upon physical examination (3 to 4 fingers below the costal margin). After omission of treatment, it returned to its prior smaller size (at the costal margin).

An additional course of amphenone in higher dosage was given two months later, the

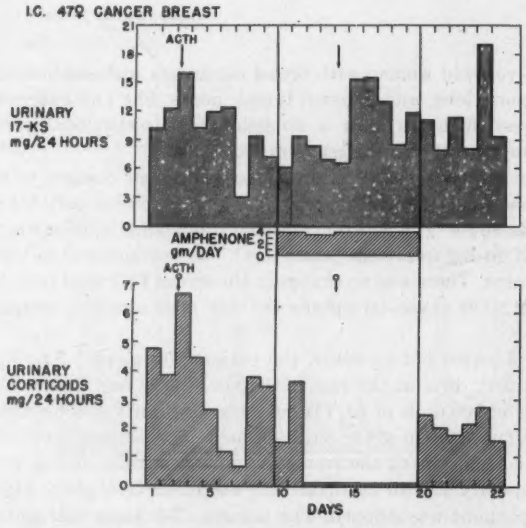

FIG. 3. I.C. (Case 4). Urinary 17-ketosteroid and 17-hydroxycorticosteroid excretion while receiving 4 grams of amphenone daily. Note suppression of the urinary corticosteroids.

results of which are shown in Figure 3. The patient received 4 grams daily—0.5 gram every three hours, for ten days. The most striking effect was the complete suppression of urinary corticosteroids beginning two days after amphenone was started and ending immediately after it was stopped. Although there was no change in the urinary corticosteroids after corticotropin (40 units intravenously over eight hours) during amphenone treatment, the serum corticosteroid level rose at this time from 16.3 to 30.4 μg. per 100 ml. and the eosinophil count fell from 53 to 8 per cu. mm. The serum electrolytes remained at a normal level during this period and the blood pressure stayed well within the normal range, although the patient appeared drowsy and pale, and complained of restlessness, weakness, and general malaise. The urinary 17-ketosteroid level did not change with amphenone, but showed the usual rise upon administration of corticotropin during the test period. The salivary Na/K ratio was 13/33 during the control period and 38/16 while the patient was receiving amphenone. A low-sodium (200 mg./day) diet had been started just prior to the pretreatment period.

The levels of serum cholesterol and PBI remained constant. The thyroidal radioiodine uptake fell from 48.8 per cent (control) to 4 per cent the second day of treatment, and rebounded to 52 per cent six days after treatment was stopped. The patient was later given sulfisoxazole (Gantrisin) with no apparent effect on the urinary corticosteroid level. The glucose tolerance curve remained normal but the urinary findings were obscured by the "3 plus" reducing agent associated with amphenone administration. There was no change in the results of liver function test. At no time during treatment with amphenone did there appear to be any effect upon the metastatic carcinoma, clinically or radiologically.

Case 5

C.B. was a 24-year-old man with classic thyrotoxicosis, untreated. The thyroidal radioiodine uptake was 95 per cent, serum PBI level 11.8 μg. per 100 ml., BMR +43 per cent, and total serum cholesterol level 120 and 130 mg. per 100 ml. He was given 2 grams of amphenone a day at first—0.5 gram every six hours. Although he said he felt calmer and slept better, the indices of thyroid function did not change significantly over a period of nine days. The dosage was then increased to 0.75 gram every six hours, for another twelve and a half days, but no further change was observed except a rise in the serum cholesterol level to 141 and 181 mg. per 100 ml. and a fall in the serum PBI level to approximately 7.0 μg. per 100 ml. on six successive occasions. The radioiodine uptake remained unchanged. There were no changes in urinary corticosteroid or 17-ketosteroid levels or in the eosinophil count, all of which remained normal. Two days after stopping amphenone, methimazole was started and the thyroidal radioiodine uptake promptly fell to zero. The effects of amphenone and thiocyanate were compared by means of a study of their effects on I^{131} uptake and release (Fig. 4, upper half). This was done ten days after starting Tapazole and the I^{131} was administered four hours after the last dose of Tapazole. The results indicated that amphenone possesses no thiocyanate-like effect on iodide trapping by the thyroid.

Case 6

J.T. was a 25-year-old Negress, with thyrotoxicosis. She had previously been treated with propylthiouracil and had had a relapse after discontinuing the drug four months before the study. Her thyroidal radioiodine uptake was 100 per cent, BMR+50 per cent, and serum PBI level 13.1 μg. per 100 ml. She was given amphenone for twenty-two days. The dosage at first was 8 grams daily—1 gram every three hours. However,

she became nauseated and weak and the dose was decreased after two and a half days to 6 grams daily—0.75 gram every three hours. The radioiodine uptake fell to 23.5 per cent on the seventh day and to 9 per cent on the tenth day of treatment. A thyroidal I^{131} uptake curve, determined on the fifteenth day, showed that thiocyanate could cause discharge of the iodide (lower portion of Fig. 4). The serum PBI level fell to 4.5 μg. per 100 ml. on the thirteenth day of treatment. There was no unequivocal clinical change.

The 17-ketosteroid excretion level did not change during amphenone therapy and the response to corticotropin (40 units of ACTH over eight hours on the seventeenth day of treatment) was unusually great—the values being 8.1 mg. for the day prior to, and 35.6 mg. for the day of, administration. The corresponding values for urinary corticosteroids

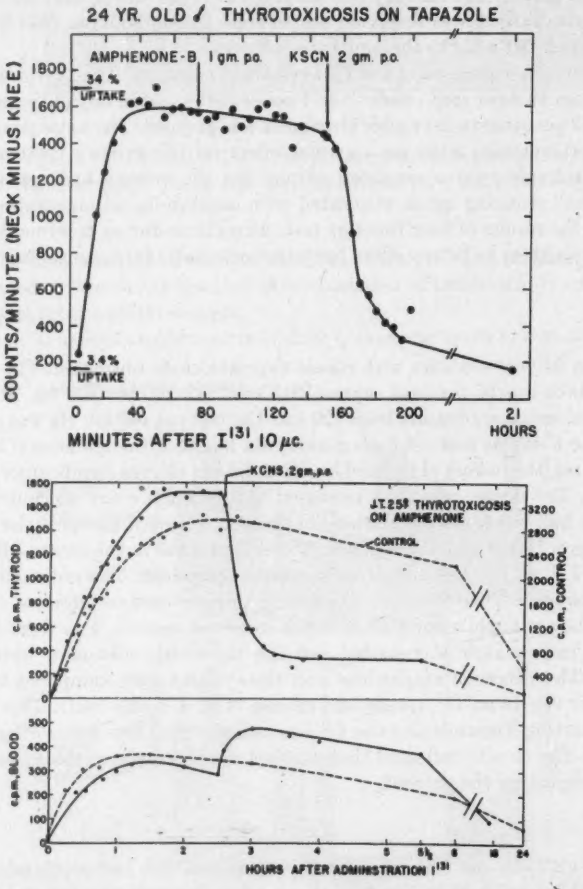

FIG. 4. Effect of amphenone on thyroid function. Upper portion of figure shows failure of amphenone to discharge iodide in Tapazole-blocked thyroid. Lower portion shows discharge of iodide by thiocyanate in amphenone-blocked gland.

were 0 and 2.48 mg. The eosinophil count changed from 405 to 23 per cu. mm.; and the serum corticosteroid level rose from 8.0 μg. per 100 ml. before corticotropin administration, to 29.6 μg. per 100 ml. afterward. The urinary corticosteroid excretion was depressed to low levels during treatment. Frequent zero levels were obtained, but the value rose to 2.48 mg. the day of corticotropin administration. Blood pressure, serum electrolyte concentration, and liver function remained normal, except for a rise in the BSP retention value to 20 per cent on the seventh day, returning to normal the fifteenth day of treatment.

A rash developed on the fifteenth day of treatment, but there was no fever. At this time the patient was receiving no drug other than amphenone and had received no other drug for a week. The rash was slightly pruritic, diffusely distributed over the trunk, arms and thighs, was finely papular, and subsided within six days despite continuance of amphenone for another seven days. Aquaphor aqua applications were the sole form of treatment.

Case 7

R.H. was a 49-year-old woman who was apparently normal in all respects, except for moderate exophthalmos and glaucoma of two years' duration. There was puffiness about

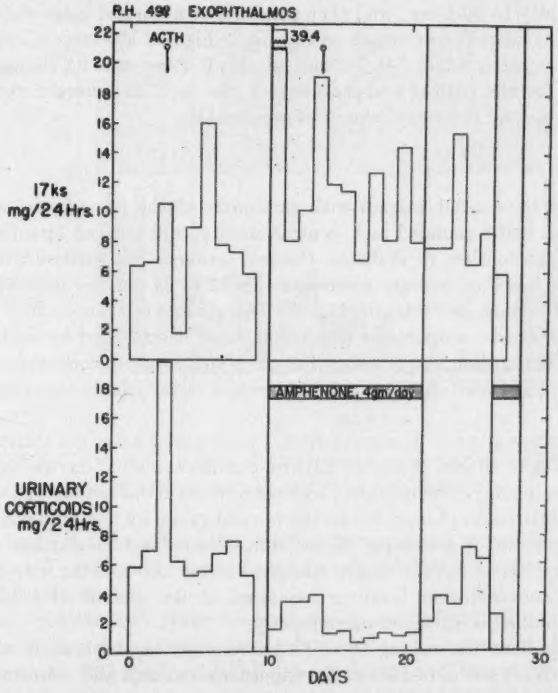

FIG. 5. R.H. (Case 7). Effect of 4 grams of amphenone daily. Note marked rise in 17-ketosteroid excretion without an associated rise in 17-hydroxycorticosteroid excretion on the first day of administration of amphenone.

the eyes and ankles. Her BMR was zero. A diagnosis of spontaneous hypothyroidism had been made twenty years before and she had been taking desiccated thyroid since that time. The dosage in recent years had been 2 grains daily. She had also been taking Lugol's solution for two years, and had had 1,600 "r" of x-ray irradiation to the pituitary for the exophthalmos, with no effect. She had never had a thyroid operation or clear signs and symptoms of thyrotoxicosis. She received 4 grams of amphenone daily for ten days—0.5 gram every three hours—and then received 3.5 and 4 grams of amphenone respectively on two days after an interim of five days. She tolerated this dosage well, except for slight heartburn which was relieved by taking milk with the amphenone.

Urinary corticosteroids were suppressed from a level of 5 to 7 mg. per day (control) to less than 1 mg. per day, with complete absence of any rise either in urinary or serum corticosteroid levels after administration of 40 units of corticotropin intravenously in eight hours (Fig. 5). Both serum and urinary corticosteroids had risen normally in response to corticotropin in the control period—serum corticosteroids from 10.3 to 20.3 μg. per 100 ml. and urinary corticosteroids from 6.9 to 19.9 mg. per day. The urinary 17-ketosteroids, however, did respond to corticotropin, both before and during amphenone administration (before amphenone—6.6 mg. (control) and 10.6 mg. with corticotropin; during amphenone—10.2 mg. and 19.3 mg., respectively). On the first day of amphenone administration the urinary volume rose to 3,300 ml., the ketosteroid excretion rose abruptly to 39.4 mg., and then subsided to a normal value the following day. The urinary corticosteroid excretion on the day of high 17-ketosteroid excretion (Fig. 5) was 1.44 mg. (control, 5.2 mg. to 7.5 mg. per day). There was no change in the BMR, exophthalmos, or the patient's appearance. A rise in 17-ketosteroid excretion did not occur with the second (two-day) course of amphenone.

Case 8

N.G. was a 35-year-old woman with carcinoma of the left adrenal and pulmonary metastases. She had a rounded face, central obesity, and marked hirsutism but did not have striae, hypertension, or diabetes. Control urinary 17-ketosteroids varied between 40 and 100 mg. per day, urinary corticosteroids 12 to 24 mg. per day, and serum corticosteroids 24 to 34 μg. per 100 ml. (Fig. 6). The glucose tolerance curve was rather flat and was unaffected by amphenone treatment. Administration of corticotropin (40 units intravenously in four hours) did not change the levels of serum corticosteroids or urinary steroids before treatment, indicating independence of the metastatic tumor from pituitary control.

Amphenone was given in dosages of from 1 to 12 grams a day for thirty-four days, the patient receiving 8, 10 and 12 grams daily on the last three full days of treatment, and 1 gram the following day. The urinary 17-ketosteroid level did not change with treatment. The urinary corticosteroid level fell to the normal range by the fifteenth day, when the patient was receiving 4 grams per day of amphenone, and fell further with increasing dosages of amphenone until it finally reached a value of 0 to 3 mg. per day. The serum corticosteroid concentration, however, remained at the control level during treatment (Fig. 6). This finding is discussed subsequently.

While the patient was taking 10 to 12 grams daily of amphenone, she was drowsy and too weak to get out of bed. She then experienced nausea and vomiting and hypotension (84/60) developed. Methemoglobinemia was evident clinically and was proved chemically. Serum electrolyte levels remained normal and the weight was constant. BSP retention rose from 14 per cent (control) to 23 per cent, and the level was 21 per

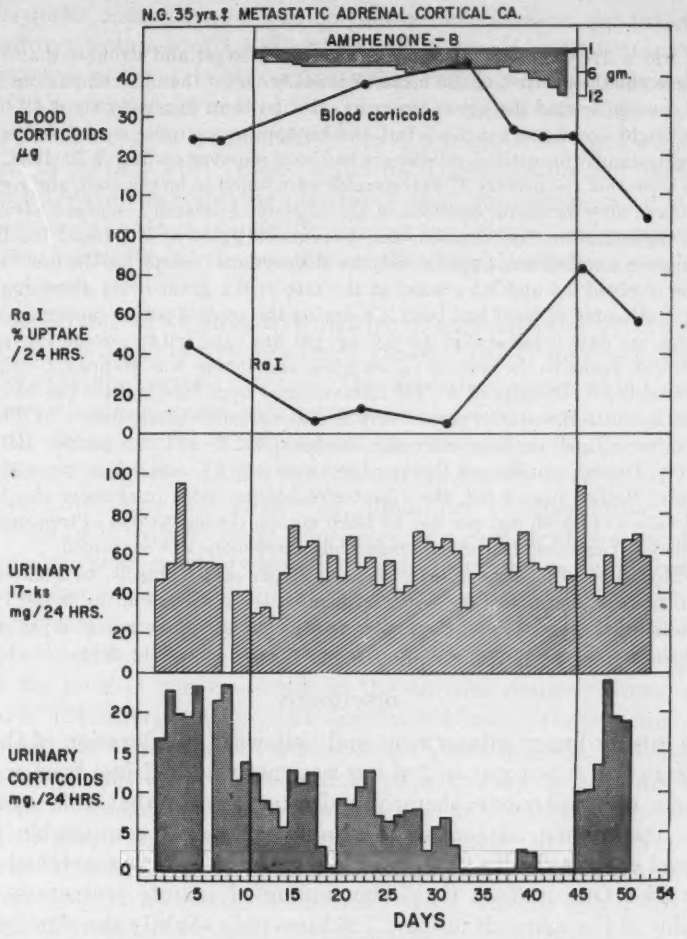

FIG. 6. N.G. (Case 8). Effect of amphenone on steroids of urine and serum, and on uptake of I^{131} by the thyroid.

cent five days later; but the other liver function tests yielded normal results, which remained unaltered. There was no change in the x-ray picture of the metastatic pulmonary lesions throughout treatment.

The thyroidal radioiodine uptake fell from 45.2 per cent (control) to 4.3 per cent on the twenty-second day of treatment, then rebounded to 82.5 per cent three days after treatment. The serum PBI level and BMR did not change and, except for lethargy, there were no clinical signs or symptoms to suggest reduced thyroid function.

Her clinical status at time of discharge on the fifty-third hospital day was the same as on admission.

Case 9

O.S. was a 29-year-old man who had been much larger and stronger than his companions in childhood. He had also matured sexually earlier than his companions and had started shaving around the age of ten years. He had been unusually short all his adult life, his height now being exactly 5 feet and his appearance quite stocky and muscular. Bilateral testicular interstitial-cell tumors had been removed on March 30, 1954. Following this operation the urinary 17-ketosteroids were found to be elevated, and roentgenograms taken after presacral injection of air suggested bilaterally enlarged adrenals. He showed pigmentation, hypotension, and weakness suggestive of adrenal insufficiency. He was given amphenone, 4 grams daily for fifteen days (except for the first two days, when he received 2.5 and 3.5 grams) at the rate of 0.5 gram every three hours. The urinary corticosteroid level had been low during the control period (approximately 0.3 to 2.0 mg. per day; occasionally, 3.0–5.0 mg. per day) and fell to zero on the tenth day of treatment, rising to the control values when amphenone was stopped. Corticotropin was administered (40 units of ACTH intravenously over four hours). The blood corticosteroid levels in the control period were as follows: immediately before ACTH, at the end of infusion, and one hour after the infusion 4.8, 2.1, and 10.8 μg. per 100 ml., respectively. During amphenone therapy they were 3.2, 3.7, and 1.5 μg. per 100 ml., respectively. Rather than a fall, the 17-ketosteroids showed a progressive rise from the control values of 35–60 mg. per day to 102.6 mg. on the eighth day of treatment, after which they fell again to the control range while amphenone was continued.

The thyroidal radioiodine uptake fell from 25 per cent (controls) to 2.6 and 2.1 per cent on the second and fifth days of treatment, with no change in the serum PBI level from the normal range. Fifteen days after treatment the uptake was 61.6 per cent. The patient showed no symptoms or signs of toxicity while taking the drug.

DISCUSSION

The extraordinary enlargement and yellowish discoloration of the adrenals observed at autopsy in 2 of our patients who had just been receiving a course of amphenone therapy indicates clearly that this compound affects the human adrenal in a manner somewhat comparable to that observed experimentally. In Cases 1 and 13 the adrenals weighed respectively 34.4 Gm. and 35 Gm. The finding of minute metastases in the medullae of the adrenals in Case 1 reduces only slightly the significance of such remarkable weights.

This gross adrenal enlargement was accompanied by significant histologic changes similar to those described by Sokoloff *et al.* (11) and O'Donnell *et al.* (12) in the adrenals of patients treated for prolonged periods with a high dosage of corticotropin.

These adrenal effects were, however, accompanied by indirect and highly variable manifestations of suppression of adrenocortical function. Thus there was a fall in urinary corticosteroid excretion in Cases 1, 4, 6, 7, 8 and 9, and a diminution or absence of a rise after corticotropin administration in Cases 1, 3, 4, 6 and 7. That these effects were not due to a reduction in total urinary output, as suggested by Marks and Leaf (13), is clear

from frequent occurrence of zero corticosteroid values in the presence of daily urinary volumes of 1,500 to 3,000 ml. (Cases 4 and 8). However, in some cases very low urinary corticosteroid values were paradoxically observed in the presence of normal blood corticosteroid levels, and even in the presence of the expected elevation of blood corticosteroid values following injection of corticotropin (Cases 4 and 6). These paradoxical findings in some cases leave room for the possibility that amphenone may actually alter the normal process of degradation and excretion of endogenous corticoids. Further studies of the possible renal or hepatic effects of amphenone are in progress.

The occurrence of oliguria, hyponatremia, azotemia and hyperkaliemia in Cases 1 and 3 and of hypotension in Cases 1 and 17 may reflect an impairment of adrenocortical function. This is further suggested in Case 3 by the failure of administered sodium to raise the serum sodium concentration until after cessation of amphenone administration. Similarly, in Case 4 the salivary ratio of sodium to potassium became reversed under the influence of amphenone (14), suggesting diminution of salt-retaining ability during treatment with the drug. Moreover, in a case of adrenal carcinoma reported elsewhere, marked reduction in urinary corticosteroid excretion during amphenone administration was associated with marked reduction in hyperglycemia and glycosuria even during reduced insulin intake (15). These observations, together with the demonstrated reduction by amphenone of the level of corticosteroids in the adrenal venous effluent of the dog, favor the interpretation that amphenone interferes in some direct manner with the formation of corticosteroids by the adrenal. The apparent irregularity of this effect in the human subject may be attributable to limitations in dosage imposed by the toxic side-effects of amphenone.

Nevertheless, a considerable degree of specificity in the effect upon corticosteroid excretion may be inferred from the dissociation of this effect from that upon 17-ketosteroid excretion, which remains either unaltered or becomes somewhat elevated during amphenone administration (Cases 7 and 9). This dissociation suggests that amphenone may act at a point in the biosynthesis of 17-hydroxycorticosteroids which is not in the direct path of the formation of those compounds contributing to the urinary excretion of 17-ketosteroids as outlined in the scheme suggested by Dorfman (16).

It should be emphasized that great care was employed to ascertain to what extent the urinary metabolites of amphenone might interfere with the chemical determination of urinary corticosteroids, particularly since the presence of such amphenone metabolites substantially increases the background color observed during such analyses. Appropriate recovery tests involving (a) the addition of known amounts of steroid to the urines

of amphenone-treated patients, (b) the addition of such urines to the urines of non-treated subjects, and (c) recovery of proportionate amounts of steroid after addition of increasing amounts of cortisone had been added to urines of amphenone-treated patients, indicated that such effects would be too small to account for the changes observed following amphenone administration. On the other hand, it is possible that suppression of urinary 17-ketosteroids might have been masked by false high values associated with amphenone administration (20).

Inhibition of thyroidal I^{131} uptake was observed in 7 of 8 patients studied in this regard. This effect is in keeping with that reported for other aniline derivatives (17). The data presented in Figure 4 indicate that amphenone has no apparent effect upon the iodide-trapping mechanism but interferes

TABLE 2. TOXIC MANIFESTATIONS IN 24 PATIENTS

Drowsiness	18
Gastro-intestinal symptoms (heartburn, nausea, vomiting)	11
Rash	6
Methemoglobinemia	6
Impaired liver function	2

only with organic binding of iodide. Accordingly, analogues of amphenone may warrant further study in relation to thyroid therapy.

Patients taking amphenone excrete in the urine a reducing substance, as yet unidentified, which appears to be a metabolite of the drug. Since this substance reacts with Benedict's reagent, it may give rise to a false positive reaction for sugar and other reducing substances. This same observation has been made on the urine of amphenone-treated rats (18).

Findings in these patients indicate that amphenone also possesses substantial toxicity, although it should be pointed out that these patients were usually in a very debilitated state. The most commonly observed toxic effects are listed in Table 2. The most frequent of these, and the chief factor limiting amphenone dosage, was drowsiness. This effect was to be anticipated from the animal studies, which had indicated that amphenone given intravenously can induce surgical anesthesia in the dog and rabbit (19). Elderly or debilitated patients appear to be more susceptible to the depressant action than more active or vigorous subjects. One patient (Case 23) became almost completely unresponsive while taking only 0.5 gram every four hours, whereas another patient (Case 13) was ambulatory while receiving 1 gram every two hours. However, interval and dosage adjustment permitted satisfactory regulation of the level of depression in any given case.

The high incidence of heartburn with frequent nausea and vomiting

may have been due to the acidity of the compound. These symptoms appear to be attributable to primary gastric irritation, since they begin shortly after ingestion of the drug and are readily relieved by ingestion of milk or antacids.

The occurrence of methemoglobinemia is to be anticipated following administration of a compound of the structure of amphenone. The methemoglobin concentration in 1 patient (Case 13) reached 15 per cent of the total hemoglobin. Methemoglobinemia per se presented no serious clinical consideration in any case, but must be regarded as clear evidence of objectionable toxicity.

The rash associated with amphenone administration was characteristically morbilliform and moderately pruritic. It usually occurred during the second or third week of therapy. Although most patients were receiving other drugs at the time, the rash occurred in 1 instance when amphenone was the only medication being given (Case 6).

Suggestive evidence of impairment of liver function was obtained in 2 patients following a course of amphenone (Cases 1 and 13). In addition, hepatomegaly developed in 1 patient (Case 4) while she was taking Amphenone; this receded when amphenone was stopped. Potential hepatic damage from compounds of this type requires careful evaluation.

In view of these multiple toxic effects of amphenone, it becomes imperative to emphasize the distinct limitations of the practical clinical potentialities of this drug. Nevertheless, its endocrinologic properties warrant further study under highly controlled clinical conditions. It is to be hoped that some of the active analogues of amphenone may prove more generally acceptable from a clinical point of view (1).

SUMMARY

Endocrinologic and toxicologic data are reported on 24 patients treated with amphenone, a compound known to inhibit both adrenal and thyroid function in several species.

Toxic side-effects, notably central nervous system depression, markedly limit the dosage which can be administered under usual clinical circumstances.

Accordingly, the marked suppression of 17-hydroxycorticosteroid production observed experimentally following amphenone administration can be induced in the human subject only with considerable irregularity. Nevertheless, 7 of 10 patients studied in this regard exhibited a decrease in urinary 17-hydroxycorticosteroid excretion and a reduction in the response to exogenous corticotropin. A comparable reduction in 17-ketosteroid excretion was not observed.

In the 2 amphenone-treated patients with advanced breast carcinoma

on whom an autopsy was performed, a two- to three-fold adrenal enlargement was observed. The enlargement was associated with histologic changes comparable to those seen after prolonged administration of corticotropin.

Amphenone therapy resulted in a striking inhibition of thyroidal I^{131} uptake in 7 of 8 patients observed for this effect.

Amphenone provides a useful tool for investigation of adrenal function under controlled clinical conditions, but its potentialities for general clinical usefulness are limited by its toxicity. Development of less toxic analogues with similar endocrinologic properties is clearly indicated, with a view to more effective pharmacologic control of endocrine function.

Acknowledgment

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A CLINICAL APPRAISAL OF RADIOIODINE TESTS OF THYROID FUNCTION*

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RADIOIODINE (I^{131}) tests, like most other diagnostic laboratory procedures, are not fully effective in separating patients with thyroid disease from normal persons. Most radioiodine diagnostic tests thus far proposed can be used with relatively high precision in the separation of patients with exophthalmic goiter[1] from normal individuals, but they are not nearly so efficient in the separation of persons with thyroid deficiency from normal persons, or in the detection of hyperthyroidism in patients who have adenomatous goiter. Thus, radioiodine tracers are more effective as diagnostic tests for certain thyroid states than for others.

Such considerations account in large part for the remarkable number and variety of procedures employing radioiodine that have been advocated for clinical diagnostic use. The diagnostic maneuvers proposed have varied from simple measurement of the excretion of radioiodine in a 24-hour or 48-hour specimen of urine to a complicated estimation of iodide clearance by the thyroid. It is to be expected, however, that no matter which radioiodine procedure is chosen, some overlap of normal and abnormal values can be anticipated, since there is no sharp dividing line between mild disease states and normality.

The present study was undertaken to evaluate simultaneously the relative merits of several practicable diagnostic radioiodine procedures in the same patients. Seven such tests were chosen (1), since many of the conflicting opinions in the literature are due to studies of only one procedure—at times, in a limited number of patients. The methods chosen were evaluated in terms of their economy, the time required, and the ease of administration and operation, as well as diagnostic precision. These were: 1) 6-hour uptake (percentage of a tracer dose collected by the thyroid in

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[1] Also known as diffuse toxic goiter, Graves' disease and Basedow's disease, as distinguished from toxic nodular goiter.

six hours); 2) 24-hour uptake (percentage of a tracer dose collected by the thyroid in twenty-four hours); 3) 24-hour excretion (percentage of radioiodine excreted into the urine in twenty-four hours); 4) accumulation rate (the proportional rate of accumulation of radioiodine by the thyroid, as described by Keating and associates (2)); 5) extrarenal disposal rate (the proportional rate of disappearance of the isotope from the blood into all organs other than the kidneys, as described by Keating and associates (2)); 6) conversion ratio (proportion of radioactivity in serum that is organically bound twenty-four hours after a tracer dose, as described by Clark and associates (3)); and 7) thyroidal iodide clearance (volume of plasma cleared of inorganic iodide by the thyroid per minute, as described by Berson and associates (4)).

MATERIAL

Tracer doses of radioiodine were given to 435 patients with various thyroid diagnoses. Forty-six of these were subsequently excluded from analysis, 15 because the thyroid diagnosis was in doubt and 31 because the behavior of radioiodine was considered to have been modified by previously administered iodides, antithyroid drugs, desiccated thyroid, or diagnostic roentgenographic contrast media containing organic iodine. The remaining 389 patients were classified as follows: *Group 1.* Euthyroid controls (181 patients). These were clinically euthyroid patients who had no thyroid complaints and whose thyroids were normal to palpation; they served as controls to define the range of normal values. *Group 2.* Diagnostic problems (38 patients). In these patients an initial suspicion of exophthalmic goiter was entertained on valid clinical grounds, but before dismissal, after employment of all clinical criteria, the patient had been judged to be euthyroid. *Group 3.* Exophthalmic goiter (92 patients). *Group 4.* Adenomatous goiter without hyperthyroidism (46 patients). *Group 5.* Adenomatous goiter with hyperthyroidism (19 patients). *Group 6.* Myxedema (13 patients). The final diagnosis of hyperthyroidism, euthyroidism or myxedema was made in every case by careful review of the record on the basis of clinical evaluation, basal metabolic rate, serum protein-bound iodine level, clinical course, and response to treatment and was entirely independent of the radioiodine tests being evaluated.

METHODS

A tracer dose of 50 microcuries of radioiodine was usually employed, although a few patients received as little as 5 microcuries or as much as 100 microcuries. Since we could determine conversion ratios accurately only if larger tracers were used, this particular test was carried out only in patients who had received tracers of 50 microcuries or more. To evaluate

thyroidal clearance by the Berson technic, radioiodine was given intravenously in the majority of cases (337 patients); the remaining 98 patients received it orally. The usual procedure was as follows: A tracer dose of radioiodine was given intravenously at 8 A.M. and a continuous *in vivo* reading was taken for thirty-five minutes over the thyroid with a scintillation counter. The thyroidal iodide clearance was then calculated as described by Berson (4). Six hours and twenty-four hours after the administration of the radioiodine, single readings were taken over the thyroid in order to determine the proportion of the dose in the gland at those intervals. Each patient was provided with six bottles for collection of urine at intervals of two, four, six, twelve, eighteen and twenty-four hours after administration of the radioiodine. From the data derived from the urinary excretion of radioiodine, the extrarenal disposal rate was calculated, and from the data derived from both the urinary excretion and *in vivo* collection of radioiodine, the thyroidal accumulation rate was calculated. Twenty-four hours after administration of the tracer, venous blood was drawn and the ratio of protein-bound radioiodine in the serum to the total radioiodine in the serum was determined (the conversion ratio).

The normal range of values for each test studied was estimated graphically by plotting a smoothed cumulative frequency-distribution curve. The intercepts at the 2.5 and 97.5 per cent levels were arbitrarily chosen as limits of the normal range.[2] Of the 181 patients composing the normal group, 27 had one or more values outside the normal range so defined. In 15 of these persons, only one tracer-test value was outside the normal range. Of the other 12 persons, there were 9 in whom two test values were outside the normal range, 2 in whom there were three such values, and 1 in whom there were five such values.

RESULTS

In Table 1 the results of the radioiodine tests in the group of controls are compared with the results in the group of patients with exophthalmic goiter. The values for protein-bound iodine and basal metabolic rate are included for reference. All of the seven radioiodine tests studied were relatively accurate for the diagnosis of exophthalmic goiter, since in relatively few instances did the values overlap those of the normal range for any test studied. Among the 92 patients with exophthalmic goiter, there were no values within the normal range for 6-hour uptake or for thyroidal iodide clearance, and only 4.8 per cent of the values were within the normal range for conversion ratio.

For the diagnosis of exophthalmic goiter in this series, the determination of 6-hour uptake and the determination of thyroidal iodide clearance were

[2] Defining a normal range which, in populations having a Gaussian distribution, would be described by $\overline{M} \pm 2\sigma$.

TABLE 1. COMPARISON OF RESULTS OF RADIOIODINE TESTS IN 181 EUTHYROID CONTROL SUBJECTS WITH RESULTS IN 92 PATIENTS WITH EXOPHTHALMIC GOITER*

Test	No thyroid disease (181 patients)		Exophthalmic goiter (92 patients)		
	Mean	95% range	Mean	Observed range	% of values in normal range
6-Hour thyroidal uptake, % of dose	14.9	7.5–25.0	59.7	33.2– 89.6	0
24-Hour thyroidal uptake, % of dose	24.8	11.9–46.5	63.6	39.9– 88.6	11.1
24-Hour urinary excretion, % of dose	58.8	36.5–74.9	23.2	6.4– 52.3	8.9
Accumulation rate, %/hour	3.3	1.1– 8.0	22.8	3.2– 59.2	11.1
Extrarenal disposal rate, %/hour	4.3	1.4– 8.4	28.3	4.8– 89.6	6.7
24-Hour conversion ratio	20.3	2.5–46.3	83.2	24.8– 99.0	4.8
Thyroidal iodide clearance, ml./min.	8.2	0 –26.0	113.5	28.3–331.5	0
Basal metabolic rate, %	−4	−20 to +16	+33	−5 to +75	11.1
Protein-bound iodine, μg./100 ml.	5.1	3.4– 7.6	12.4	6.0– 20.2	3.9

* In this and subsequent tables, data on basal metabolic rate and serum protein-bound iodine are included for reference.

the most precise of the tests studied, since they distinguished sharply between the group of patients with exophthalmic goiter and the group in whom there was no thyroid disease. Since the determination of thyroidal iodide clearance requires intravenous administration of the isotope and the careful attention of trained personnel for accurate measurement and calculation of results, it is less adapted for use on a large scale than is the determination of 6-hour uptake.

The 24-hour conversion ratio also was very precise in the diagnosis of exophthalmic goiter, with minimal overlap of values between the group of persons with hyperthyroidism and the group without thyroid disease. An objection to use of the conversion ratio is the requirement of a relatively large tracer dose of radioiodine.[3] No matter how sensitive the radiation-

[3] With present equipment, the conversion ratio can be determined accurately in exophthalmic goiter with a dose as small as 20 to 25 microcuries, but to determine it precisely in patients with myxedema may require 100 microcuries.

detection equipment employed in radioiodine tracer tests, detection of a minute fraction of a dose in a small quantity of blood, as in determination of the conversion ratio, will always of necessity require a larger quantity of radioactivity than is needed to detect (with similar apparatus) the much larger proportion of the dose in urine or in the thyroid. Since, for routine clinical use, it is desirable to keep the tracer dose as minute as possible, and since other comparably accurate radioiodine tests may be readily performed with tracer doses of 5 microcuries or less, we have not felt that it is justifiable to determine the conversion ratio on a routine basis.

Determination of the 24-hour (or 48-hour) excretion of radioiodine into the urine is a simple test, and it usually furnishes a fairly accurate portrayal of thyroid function. Skanse (5), Arnott and associates (6), and Keating and associates (2) studied this test carefully and defined its limitations. Its principal disadvantage is its dependence upon the full co-operation of the patient; if the collections of urine are incomplete, results are misleading. In addition, in patients with renal disease or congestive heart failure, the values for urinary excretion of the isotope may give a false impression about thyroid function. For these reasons, this and other urinary tests are now used infrequently.

Determination of thyroidal accumulation rate and determination of extrarenal disposal rate are also dependent on co-operation of the patients, since the first test is based in part, and the latter entirely, on data derived from determinations of fractional urinary excretion. Partly because of this, but particularly because of their complexity, these tests are used infrequently.

The radioiodine test that is perhaps most widely used throughout the country at present as a diagnostic aid is that of the 24-hour uptake by the thyroid. It is simple to perform and requires minimal co-operation by the patient and a minimal dose of radioiodine. Werner and associates (7) evaluated this procedure in 1,400 patients and found that in 90 per cent of euthyroid individuals the 24-hour uptake was between 10 and 40 per cent. They also found that in 11 per cent of hyperthyroid subjects the uptake was less than 40 per cent. Our findings (11.1 per cent within the normal range) were similar.

The test for 6-hour uptake proved to be better than the test for 24-hour uptake, since it was completely effective in separating the persons who were euthyroid from those who had exophthalmic goiter, including the 11.1 per cent in whom the 24-hour uptake fell within the normal range. The normal range of values for the control group was 7.5 to 25 per cent, whereas the range observed in patients with exophthalmic goiter was 33.2 to 89.6 per cent. One might anticipate that the 6-hour *in vivo* collection

of radioiodine by the thyroid would represent a more accurate diagnostic test than would the 24-hour collection, since in most patients with exophthalmic goiter the peak collection of the isotope occurs much earlier than twenty-four hours after administration of the tracer. Besides being one of the most accurate of the radioiodine tests, the determination of 6-hour thyroidal collection also has the advantages of being quick, simple, and inexpensive and of requiring relatively less radioactivity.

Some workers have proposed, as a preferable diagnostic test, the determination of radioiodine collection by the thyroid one, two, or three hours after the administration of the tracer. From theoretical considerations, such time intervals might well prove equally satisfactory, although we have not had experience with them. A 6-hour interval was chosen simply because this was a most convenient one for our particular needs.

TABLE 2. COMPARISON OF RESULTS OF RADIOIODINE TESTS IN 181 EUTHYROID CONTROL SUBJECTS WITH RESULTS IN A GROUP OF 58 PATIENTS SUSPECTED OF HAVING HYPERTHYROIDISM (20 OF WHOM WERE DETERMINED TO HAVE EXOPHTHALMIC GOITER AND 38 OF WHOM WERE DETERMINED TO BE EUTHYROID)

Test	No thyroid disease (181 patients)		Diagnostically difficult exophthalmic goiter (20 patients)			Euthyroid patients suspected of having exophthalmic goiter (38 patients)		
	Mean	95% range	Mean	Observed range	% of values in normal range	Mean	Observed range	% of values in normal range*
6-Hour thyroidal uptake, % of dose	14.9	7.5–25.0	59.1	35.1– 78.0	0	16.1	6.3–31.9	95.8
24-Hour thyroidal uptake, % of dose	24.8	11.9–46.5	64.4	44.2– 88.6	10.0	24.5	7.2–45.4	100.0
24-Hour urinary excretion, % of dose	58.8	36.5–74.9	25.3	6.8– 48.6	0	59.5	37.7–83.5	100.0
Accumulation rate, %/hour	3.3	1.1– 8.0	21.9	8.2– 40.2	0	3.5	0.7– 8.1	97.3
Extrarenal disposal rate, %/hour	4.3	1.4– 8.4	21.9	9.1– 55.5	0	5.0	0.3–10.0	81.6
24-Hour conversion ratio	20.3	2.5–46.3	82.2	40.3– 97.8	7.2	22.2	6.1–55.9	96.9
Thyroidal iodide clearance, ml./min.	8.2	0–26.0	100.7	33.1–233.2	0	10.0	0–25.2	100.0
Basal metabolic rate, %	−4	−20 to +16	+30	−5 to +60	15.0	+13	−16 to +65	56.8
Protein-bound iodine, μg./100 ml. of serum	5.1	3.4– 7.6	11.2	6.0– 19.7	10.5	6.5	3.6– 7.9	94.5

* In a few instances the values obtained with the radioiodine tests in the euthyroid persons were within the hypothyroid range, but since the patients in question were suspected of being hyperthyroid, such low values did not weigh against the diagnostic accuracy of the test as used.

Further insight into the accuracy of the various radioiodine tests is afforded by observing the results obtained in a group of 58 patients in whom the thyroid diagnosis was originally in doubt. Twenty of these patients were subsequently proved to have exophthalmic goiter (and, therefore, have also been included in group 3, that is, the previously discussed group of 92 patients with exophthalmic goiter). The remaining 38 comprise group 2. These 38 patients had been suspected of being hyperthyroid, but were, in the final appraisal, considered to be euthyroid. The diagnosis of hyperthyroidism or euthyroidism was made, as previously stated, in every one of the 58 cases, without reference to the radioiodine tests that were being evaluated. In Table 2 the results of the various radioiodine tests in these 58 patients, regrouped as just described, are compared with the results in euthyroid controls. The basal metabolic rate and the values for serum protein-bound iodine concentration are included for reference. As seen in this table, the tests for 6-hour thyroidal uptake, the test for thyroidal iodide clearance, and the conversion ratio were of equal diagnostic accuracy in indicating the presence of hyperthyroidism in the group of 20 patients who subsequently proved to have exophthalmic goiter, and in indicating the absence of hyperthyroidism in the group of 38 patients who subsequently were considered to be euthyroid. For the latter group, it should be noted that the average value for serum protein-bound iodine concentration was 1.4 micrograms above the mean normal for the euthyroid group of 181 patients and that the average basal metabolic rate was +13 per cent. Although these values may, in part, reflect misleading laboratory data which led to an unsupported presumption of exophthalmic goiter in some of these instances, one must acknowledge that the group may contain one or more patients who, followed long enough, would prove to have exophthalmic goiter. No matter how much care be taken it is never possible to be entirely secure about the absence of hyperthyroidism in patients suspected of having it. On this account the data comprising Table 2 must be interpreted with some caution.

The decision as to whether a patient who has a nodular goiter is or is not hyperthyroid may be very difficult. In such a situation, the clinician often needs all the laboratory diagnostic aids he can muster to arrive at a definite conclusion but, unfortunately, the results of radioiodine tests are much less decisive here than in the diagnosis of exophthalmic goiter. Although the results of such tests are usually within the normal range in patients with nodular goiters who are euthyroid, they also are frequently within normal limits in patients who have toxic nodular goiters. As seen in Table 3, the results of radioiodine tests were appropriately within the normal range in the 46 patients who had adenomatous goiter without hyperthyroidism. However, in the 19 patients who had adenomatous goiter

TABLE 3. COMPARISON OF RESULTS OF RADIOIODINE TESTS IN 181 EUTHYROID CONTROL SUBJECTS WITH RESULTS IN 46 PATIENTS WHO HAD ADENOMATOUS GOITER WITHOUT HYPERTHYROIDISM AND 19 PATIENTS WHO HAD ADENOMATOUS GOITER WITH HYPERTHYROIDISM

Test	No thyroid disease (181 patients)		Adenomatous goiter without hyperthyroidism (46 patients)			Adenomatous goiter with hyperthyroidism (19 patients)		
	Mean	95% range	Mean	Observed range	% of values in normal range*	Mean	Observed range	% of values in normal range*
6-Hour thyroidal uptake, % of dose	14.9	7.5–25.0	15.5	4.4–27.7	93.9	21.2	5.7–44.5	58.3
24-Hour thyroidal uptake, % of dose	24.8	11.9–46.5	25.3	6.8–44.1	100.0	32.9	8.0–51.3	77.8
24-Hour urinary excretion, % of dose	58.8	36.5–74.9	54.4	26.0–76.3	97.8	40.6	8.1–73.7	56.3
Accumulation rate, %/hour	3.3	1.1– 8.0	3.6	0.8– 9.0	95.6	4.9	0.7–13.4	80.0
Extrarenal disposal rate, %/hour	4.3	1.4– 8.4	5.8	1.8–21.2	80.0	8.2	1.4–24.5	73.3
24-Hour conversion ratio	20.3	2.5–46.3	16.2	6.7–47.1	97.6	43.1	6.2–92.3	58.8
Thyroidal iodide clearance, ml./min.	8.2	0–26.0	8.8	0.5–30.8	95.4	21.2	0–69.9	68.8
Basal metabolic rate, %	−4	−20 to +16	+2	−17 to +22	93.5	+23	+12 to +35	18.7
Protein-bound iodine, μg./100 ml. of serum	5.1	3.4– 7.6	5.7	1.5– 7.4	100.0	9.4	3.9–16.2	22.2

* In a few instances the values obtained with the radioiodine tests in the patients with adenomatous goiters were within the hypothyroid range, but since the question to be settled in these patients was whether they were or were not hyperthyroid, such low values did not weigh against the diagnostic accuracy of the test as used.

with hyperthyroidism the results of the 7 radioiodine tests being evaluated were within the hyperthyroid range in only 7 cases for the "best" test and in only 3 cases for the "worst" test.

Likewise, radioiodine tests are of limited usefulness in the diagnosis of myxedema (Table 4). Determinations of thyroidal iodide clearance and conversion ratio are quite useless in this regard, since the euthyroid range in these tests overlaps completely the myxedematous range. In our hands the clearance test has proved insensitive and inaccurate at low levels. Determination of 24-hour urinary excretion of radioiodine is virtually useless in the diagnosis of myxedema, since the results obtained largely overlap those obtained for the euthyroid group. However, the determination of 6-hour uptake or 24-hour uptake, and particularly the former, is of considerable discriminatory value in the diagnosis of myxedema. In all myxe-

TABLE 4. COMPARISON OF THE RESULTS OF RADIOIODINE TESTS IN 181 EUTHYROID CONTROL SUBJECTS WITH THE RESULTS IN 13 PATIENTS WITH MYXEDEMA

Test	No thyroid disease (181 patients)		Myxedema (13 patients)		
	Mean	95% range	Mean	Observed range	% of values in normal range
6-Hour thyroidal uptake, % of dose	14.9	7.5–25.0	4.1	1.7– 6.7	0
24-Hour thyroidal uptake, % of dose	24.8	11.9–46.5	5.5	0.6–18.1	7.7
24-Hour urinary excretion, % of dose	58.8	36.5–74.9	65.5	52.8–79.8	91.7
Accumulation rate, %/hour	3.3	1.1– 8.0	0.6	0.1– 1.8	16.6
Extrarenal disposal rate, %/hour	4.3	1.4– 8.4	1.7	0.8– 3.3	58.3
24-Hour conversion ratio	20.3	2.5–46.3	7.3	3.2–10.2	100.0
Thyroidal iodide clearance, ml./min.	8.2	0 –26.0	1.2	0 – 5.7	100.0
Basal metabolic rate, %	−4	−20 to +16	−26	−40 to −13	15.4
Protein-bound iodine, μg./100 ml. of serum	5.1	3.4– 7.6	1.0	0.2– 2.2	0

dematous patients studied, the thyroidal 6-hour uptake of radioiodine was 6.7 per cent or less, whereas control values were 7.5 per cent of the dose or more. Nevertheless, clinical interpretation of abnormally low uptakes is always hindered by the knowledge that exogenous iodine from various sources, known and unknown, frequently is the cause of such findings in normal or hyperthyroid persons.

The degree of abnormality indicated by the results of a radioiodine test often is not correlated with the severity of hyperthyroidism as estimated by the physician caring for the patient, whereas elevation of the basal metabolic rate usually does correlate better with clinical severity. On this account, it is of interest to note that in the patients with exophthalmic goiter there was little correlation between the degree of elevation of the basal metabolic rate and the degree to which the results of the radioiodine tests were abnormal. Figure 1 illustrates this for the 24-hour uptake. The coefficients of correlation between the basal metabolic rates and the results of the four radioiodine tests so analyzed are shown in Table 5; they obviously are low. Although the patient may be mildly hyperthyroid,

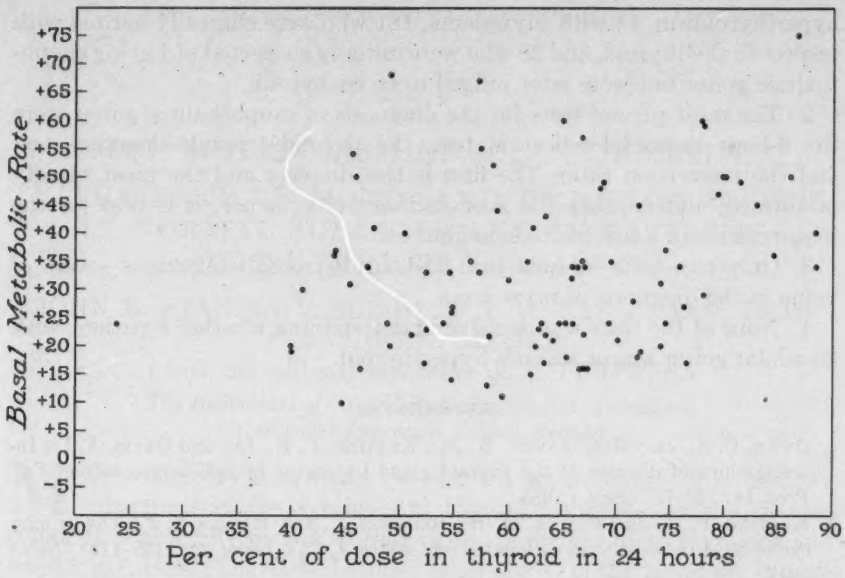

FIG. 1. Scatter diagram correlating basal metabolic rate with 24-hour thyroidal uptake of I^{131} in patients with exophthalmic goiter. It is evident that no good correlation exists between these indices of thyroid function in this group of patients.

with a basal metabolic rate that is nearly normal, the values for the radioiodine tests are usually far above the normal range and thus are diagnostically decisive in a patient whose hyperthyroid status is completely equivocal by other criteria.

SUMMARY

1. Seven types of diagnostic radioiodine (I^{131}) tests were compared simultaneously in 92 patients with exophthalmic goiter, 46 with adenomatous goiter without hyperthyroidism, 19 with adenomatous goiter and

TABLE 5. COEFFICIENTS OF CORRELATION BETWEEN BASAL METABOLIC RATES AND RESULTS OF RADIOIODINE TESTS IN THE 92 PATIENTS WITH EXOPHTHALMIC GOITER

Variables correlated	Correlation
Basal metabolic rate and 6-hour thyroidal uptake	0.23 ± 0.13
Basal metabolic rate and 24-hour thyroidal uptake	0.23 ± 0.10
Basal metabolic rate and 24-hour conversion ratio	0.10 ± 0.12
Basal metabolic rate and thyroidal iodide clearance	0.18 ± 0.12

hyperthyroidism, 13 with myxedema, 181 who were clinically normal with respect to the thyroid, and 38 who were initially suspected of having exophthalmic goiter but were later judged to be euthyroid.

2. The most precise tests for the diagnosis of exophthalmic goiter were the 6-hour thyroidal-collection test, the thyroidal iodide-clearance test and the conversion ratio. The first is the simplest and the most rapidly performed, and requires the least radioactivity; hence, it is best for the requirements of a routine radioiodine test.

3. Only two tests—6-hour and 24-hour thyroidal collections—were of value in the diagnosis of myxedema.

4. None of the tests was decisive in determining whether a patient with a nodular goiter was or was not hyperthyroid.

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THE METABOLISM OF IODOTYROSINES.

I. THE FATE OF MONO- AND DI-IODOTYROSINE IN NORMAL SUBJECTS AND IN PATIENTS WITH VARIOUS DISEASES*

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MONO- and di-iodotyrosine (T_1 and T_2) are important intermediary substances in the synthesis of thyroid hormones by the thyroid gland. It is generally presumed that these are precursors of thyroxine and tri-iodothyronine. The T_1 and almost all of the T_2 of the gland are in peptide linkage in the thyroglobulin (1). Normally they do not escape into the general circulation. There has been little reason to suppose that these two substances play a role in the metabolism of iodine outside the thyroid gland.

Almost all recent studies have failed to demonstrate either T_1 or T_2 in the peripheral blood of normal subjects or of those with thyroid disease (2–6). Robbins, Rall and Rawson (7) have demonstrated a protein complex in the blood of certain patients with cancer of the thyroid which yields T_1 on hydrolysis, and Robbins *et al.* (8) and Tong, Taurog and Chaikoff (9) have found protein complexes in the blood after administration of large doses of I^{131} which yield T_1 and T_2 upon hydrolysis. These findings have been attributed to radiation effect. Benua and Dobyns (10) observed that minute amounts of labeled[1] T_1* and T_2* appeared in chromatograms of the serum of patients who had received therapeutic amounts of I^{131} for treatment of thyroid cancer or thyrotoxicosis. The early appearance of T_2* could not be attributed to radiation effect. The chromatograms were prepared in only a single solvent system.

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[1] A labeled compound is indicated by an asterisk, *e.g.*, T_2* = di-iodotyrosine labeled with I^{131}.

The disposal of T_2* has been carefully studied in the rat by Johnson and Albert (11) and by Tong, Taurog and Chaikoff (12), and similarly in 3 myxedematous patients treated with desiccated thyroid by Albert and Keating (13). Only a small fraction of injected T_2* appeared in the urine unchanged; almost all was recoverable from the urine as iodide. In the rat, chromatographic analyses of sera indicated the transient appearance of several iodinated derivatives of tyrosine (12).

In 1953, Costa *et al.* (14) reported that chromatograms from sera of 2 patients with "endemic cretinism" who had been given I^{131} displayed zones of labeled iodine[2] corresponding to di-iodotyrosine.[3] More recently the authors have observed mono- and di-iodotyrosine in the blood of a patient with familial goiter and hypothyroidism (15). These findings, together with additional observations upon our patient with familial goiter and similar subjects (16, 17), have necessitated further examination of the normal disposal of labeled mono- and di-iodotyrosine in man.

CLINICAL MATERIAL AND METHODS

Subjects

Patients were selected from the wards and the ambulatory clinic of the Academisch Ziekenhuis, Leiden. Several were in the hospital for possible endocrinologic disorders which were subsequently ruled out. Two were diabetic and 3 had pathologically undefined enlargement of the thyroid which fulfilled the clinical criteria for "simple goiter." Significant endocrine disease was not detected in the remaining patients. Each patient was given an intravenous injection of T_1* or T_2*, with a known or subsequently measured amount of carrier T_1 or T_2, in a total volume of 8–10 ml. of solution. An aliquot was saved for a standard and for chromatographic analysis. Urine was collected without catheterization.

Preparation of labeled T_2

Labeled *dl*-di-iodotyrosine was prepared by exchange, as described by Tong, Taurog and Chaikoff (12). In most of the experiments an aliquot of the stock solution of T_2* was diluted with saline containing carrier T_2, sterilized for thirty minutes at 100°C. and injected. Separation was in a butanol-acetic acid solvent system (2). In a few experiments T_2* for injection was purified by elution from chromatography paper after separation in a butanol-acetic acid solvent system. Subsequently the solutions were analyzed by the method of Barker (18) for content of I^{127}, and the quantity of carrier T_2 calculated. The carrier T_2 varied in different experiments from 78 to 1,000 μg. The quantity of injected I^{131} varied from 20 to 50 μc. for each dose. The preparation of T_2* purified chromatographically had the advantage of being more than 95 per cent pure, but suffered the disadvantage that the amount of carrier T_2 could not be known exactly until the completion of the tedious I^{127} analysis.

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[2] The term "labeled iodine" is used in the generic sense to include iodide and all compounds of iodine which may be marked with I^{131}.

[3] This important observation was overlooked in an earlier publication from this laboratory (15).

*Preparation of dl- and l-T_1**

These substances were prepared by iodinization of racemic or levorotatory tyrosine,[4] as described by Roche *et al.* (19). The prepared T_1* was purified by elution, as previously described for the purification of T_2*. This method yielded preparations of T_1* of 97 per cent purity and better. *l*-Mono-iodotyrosine is optically stable at the temperature of boiling water (20).

A preparation of *l*-T_1 was made in the same manner, except that a larger amount of iodide was used and no I^{131} was added. The product was diluted to a 5 per cent solution, and in a Zeiss polarimeter had a specific rotation $(\alpha)_D^{20} = -3.4$ before sterilization and $(\alpha)_D^{20} = -3.6$ after sterilization. Harington and Pitt-Rivers (20) found that their preparation of *l*-mono-iodotyrosine had a specific rotation $(\alpha)_D^{20} = -4.4$.

Preparation of samples and chromatography

Samples of urine were brought to the laboratory immediately after collection. The radioactivity was estimated roughly, and an aliquot selected which would contain between 5,000 and 20,000 counts per minute as recorded in a well-type scintillation crystal detector. This counting rate was usually provided by 0.1 to 0.5 ml. of urine. Carriers of approximately 100 μg. each of iodide, T_1, T_2, tri-iodothyronine and thyroxine were added from an ethanol-ammonia solution, and the sample applied in a thin line (3 to 5 cm. long) to Whatman #3 paper. The paper was cut in long tails for each sample. Occasionally an amount of urine greater than 0.5 ml. was required to provide the necessary number of counts. If so, 2 ml. of the sample was acidified with H_2SO_4 to pH 2 and extracted first with 7 ml. and then twice with 5 ml. of *n*-butanol which was equilibrated with 5 per cent $Na_2S_2O_3$ solution. The extracts were pooled, made alkaline with ammonia, and evaporated to dryness in an air stream at 60° C. The residue was taken up with a few drops of ethanol-NH_3 and applied as before to chromatography paper.

Descending phase chromatography was used throughout. When the solvent front had progressed 35 to 40 cm. beyond the origin, the paper strips were removed from the tank, dried, neutralized with a spray of 2.5 per cent K_2CO_3 solution, and developed with diazotized sulfanilic acid spray. Iodide was developed by spraying with 1 per cent palladium chloride. When dry, the papers were cut in strips 1 cm. wide for counting in the well-type scintillation crystal detector. The quantity of labeled iodine present was always referred to the dose given the patient by counting an appropriate standard, and an aliquot of the blood serum or urine, or both. In this way it was possible to estimate total recovery of labeled iodine from the paper strip.

In almost all strips there were a few counts above background in the paper between zones demarcated by the carriers. The contribution of this "smeared" radioactivity to that measured in the iodide and T_2* zones could not be assessed with certainty. The following conventions were employed: It was assumed that the "smeared activity" was uniform throughout the paper. This seemed reasonable since, except in the iodide and T_2 zones, the counts above background were fairly uniformly distributed from one end of the strip to the other. A mean was obtained of the counting rate of the labeled iodine in those centimeter segments of the paper which were no closer than 1 cm. to a stained carrier zone. A value for the "smearing" assumed to be present in the iodide and T_2* zones was calculated and subtracted. The mean correction for smearing was 0.32 per cent of the applied labeled iodine per centimeter. After correcting for smearing the mean recovery of labeled iodine in the T_2* and iodide zones was 89.8 per cent of the total

[4] Supplied by the Department of Organic Chemistry, University of Leiden.

labeled iodine in the urine applied directly to the paper. When butanol extracts of urine were used (9 of the 46 chromatograms of Table 1), the recovery was slightly less.

A 2-ml. sample of serum was extracted in glass-stoppered centrifuge tubes. The samples were acidified to pH 2 with H_2SO_4 and extracted with 7 ml., 5 ml. and 5 ml. of *n*-butanol in equilibrium with 5 per cent $Na_2S_2O_3$. The washings were pooled and treated as described for the urine samples.

RESULTS

Control observations

I^{131} was administered orally in therapeutic amounts to 2 thyrotoxic patients, to 1 normal subject intravenously in a dose of 20 μc., and to 2 patients with enlarged thyroids of undetermined type. Serum and urine samples obtained at various times up to twenty-four hours failed to show evidence of significant amounts of T_1* or T_2*.

Samples of T_2* were added to normal urine and allowed to incubate for four hours at room temperature and at 37°. Chromatographic analysis failed to show any deiodination of the T_2*. Samples of urine kept for many days at ice-box temperature yielded the same distribution of components upon chromatography as did the sample initially, and with excellent quantitative agreement when correction was made for radioactive decay. Labeled di-iodotyrosine and I^{131} were recovered unchanged when added to serum *in vitro*.

Di-iodotyrosine

The pattern of distribution of labeled iodine in successive urine samples after intravenous administration of T_2* is illustrated in Figure 1. The patient was given an injection of labeled iodine which, by chromatographic analysis, was 77.0 per cent T_2* and the rest iodide. Almost all of the labeled iodine in the urine appeared as iodide. The fraction of the labeled iodine present as T_2* decreased with successive urine samples, and was vanishingly small after the fourth hour. Figure 1 is constructed to show the percentage of the administered dose excreted in the various zones of the chromatograms during successive collection periods. The bottom chart is from a chromatogram of a two-hour collection period, whereas the others are for collection periods of one hour each.

The excretion of labeled iodine after intravenous injection of T_2* was measured in 16 studies on 15 patients. The data appear in Table 1. The last three columns show the percentage of the actual amount of injected T_2* which was excreted intact; these values have been corrected for the contaminating iodide which was present in the injection solution.

Only a small fraction of injected T_2* was excreted intact. The labeled iodine which was present as T_2* in the urine of the first hour was more than

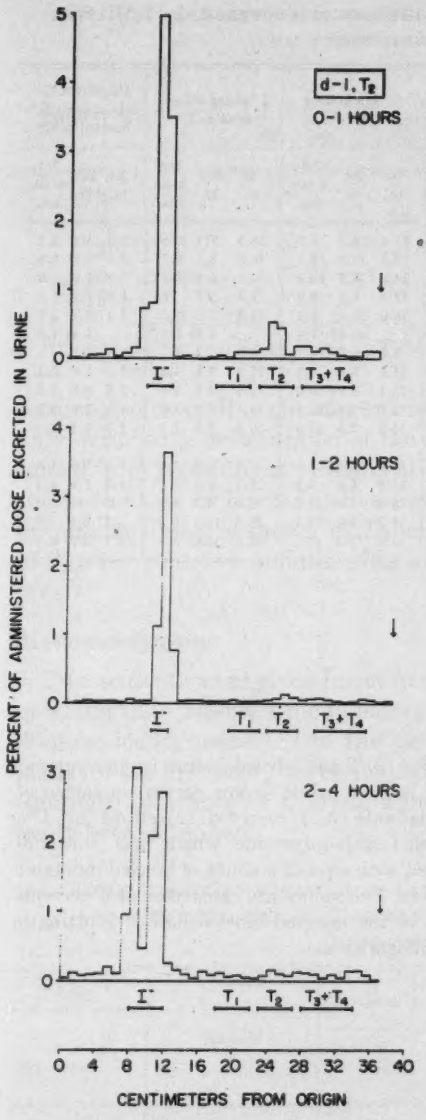

FIG. 1. Chromatograms obtained on urine specimens after intravenous administration of *dl*-T_2* (labeled di-iodotyrosine) to a patient with mild bronchial asthma. A butanol-acetic acid solvent system was used. The arrows are at the solvent front. I^- = iodide; T_1 = mono-iodotyrosine; T_2 = di-iodotyrosine; T_3 = tri-iodothyronine; T_4 = thyroxine. The positions of the designated zones were obtained by staining carriers added to the specimen prior to application to the chromatography paper.

15 per cent of the dose in only 2 patients; and in only 2 patients was it more than 5 per cent in the urine collected between two and four hours. In none of the chromatograms was there significant labeled iodine in any zone other than those of iodide and T_2. If conjugation or degradation products were formed, these were in a concentration too small to be detected.

Only 1 patient excreted more than 4 per cent of the injected T_2* as T_2* during the four hours after administration. This patient, a severe diabetic, was first studied early in the course of these experiments (Table 1, #15, a). When it was observed that the fraction of the dose excreted as T_2* was about twice as great as in the other patients, the study was repeated, using a smaller amount of carrier T_2. This was done on the supposition that the carrier T_2 in the first experiment might have saturated a deiodinating mechanism. The results, however, were similar (Table 1, #15, b). All urine specimens showed a strongly positive reaction for reducing substance with Benedict's reagent. Urine volumes were much smaller than in several other patients. The rate of urine formation, therefore, was not the controlling factor in the excessive excretion rate of T_2* by this patient.

TABLE 1. THE URINARY EXCRETION OF I^{131}-LABELED DI-IODOTYROSINE (T_2*) AFTER INTRAVENOUS INJECTION

Patient No.	Age	Sex	Diagnosis	Dose		% Dose in urine as T_3*			% Labeled iodine in urine as T_3*			Cumulative % administered T_3* excreted as T_3*		
				% T_3*	Carrier T_3 (μg.)	1st hr.	2nd hr.	3rd & 4th hrs.	1st hr.	2nd hr.	3rd & 4th hrs.	1st hr.	2nd hr.	3rd & 4th hrs.
1	26	F	? Cushing's disease	91.2	1,000	11.5	14.2	8.9	18.1	7.4	2.6	2.3	3.4	3.7
2	56	F	? Addison's disease	82.3	1,000	13.8	10.8	18.1	14.2	6.1	2.7	2.4	3.2	3.8
3	36	M	? Addison's disease	79.0	500	14.3	8.8	12.4	4.1	2.4	1.7	.8	1.0	1.3
4	26	M	Testicular deficiency	89.2	502	12.7	4.3	9.9	7.7	3.7	.8	1.0	1.2	1.3
5	28	F	Hirsutism	88.8	400	16.0	10.9	9.2	13.5	2.3	1.5	2.4	2.7	2.9
6	20	M	Neuritis	92.1	100		20.0†	7.8		6.4†	1.3		1.4†	1.7
7	57	M	Gynecomastia	95.3	100	2.8	9.0	♯	8.7	11.3	♯	.3	1.3	♯
8	40	F	Simple goiter	99.2	100	11.2	5.5	4.2	11.2	5.5	4.2	1.3	1.8	2.1
9	24	M	Gastritis	83.8	100	13.4	9.7	10.0	12.6	4.1	8.6	2.0	2.5	3.6
10	48	M	Pericardial cyst	77.0	100	9.7	7.9	13.7	10.1	7.0	3.2	1.3	2.0	2.5
11	36	F	Asthma	77.0	100	13.4	7.8	12.6	9.8	3.5	1.8	1.7	2.1	2.4
12	31	M	Cushing's disease, postoperative	84.4	96	15.6	8.4	14.1	7.0	2.2	2.4	1.2	1.5	1.9
13	47	F	Simple goiter	97.2	78	12.0	5.4	8.2	12.3	4.1	4.0	1.5	1.8	2.1
14	15	F	Simple goiter	96.6	78	13.9	4.8	3.9	2.0	4.7	3.3	.3	.5	.7
15 (a)	41	F	Diabetes	88.4	493	19.2	9.4	12.1	23.6	11.1	6.9	5.2	6.4	7.3
15 (b)	Idem	Idem	Idem	96.9	78	13.4	11.3	11.1	26.5	15.0	5.9	3.7	5.5	6.1

† First and second hours.
♯ Eloped at 2 hours.

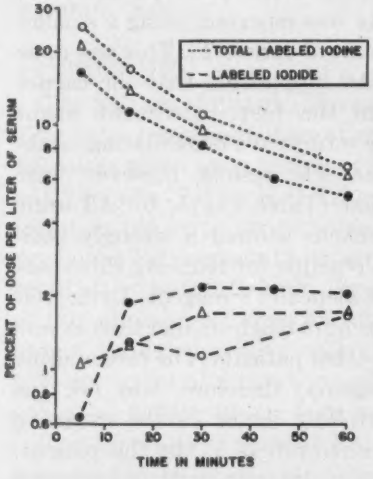

FIG. 2. Total labeled iodine (upper curves) and labeled iodide (lower curves) in serum of 3 patients who received injections of I^{131}-labeled di-iodotyrosine which was contaminated with a small amount of labeled inorganic iodide. The values are calculated as a percentage of the injected labeled iodine. Plotting is semilogarithmic.

Two patients, #2 and #12, were given 1 gram daily of potassium iodide for three days preceding the injection of T_2*. The results did not differ appreciably from the others.

Serial serum samples were obtained frequently during the first hour after administration of T_2* to 3 patients. The results appear in Figure 2. In all 3 studies there was a rise in the concentration of labeled iodide in the

TABLE 2. LABELED IODINE IN THE SERUM AS T_2* AFTER INTRAVENOUS ADMINISTRATION OF I^{131}-LABELED *dl*-T_2*

Patient No.*	% T_2* in injection solution	% Labeled iodine in serum as T_2*	
		5 mins.	60 mins.
3	79.0	90.8	60.8
5	88.8	92.6	56.4
8	99.2	95.5	74.5
13	97.2	94.5	66.5
15	96.9	95.5	74.5

* Numbers correspond to those in Table 1.

serum despite a fall in the concentration of total labeled iodine. This could only occur with deiodination of the injected T_2*. Serum samples were obtained from 2 additional patients at five and sixty minutes. The change in the percentage of labeled iodine present in the serum as T_2* (chromatographic analysis) is shown in Table 2. The proportion fell from a mean of 93.8 per cent five minutes after injection to 66.5 per cent at sixty minutes.

Mono-iodotyrosine

Two patients were given injections of labeled *dl*-mono-iodotyrosine and, at a later date, labeled *l*-mono-iodotyrosine. A third patient was given only *dl*-mono-iodotyrosine. As in the previous studies, urine collections were made for the first hour, the second hour, and for the third and fourth hours combined. Methods of analysis were identical with those which have already been described.

TABLE 3. URINARY EXCRETION OF LABELED INORGANIC IODIDE AND LABELED MONO-IODOTYROSINE (T_1*) AFTER INJECTION OF I^{131}-LABELED *l*- AND *dl*-MONO-IODOTYROSINE

Patient			Diagnosis	T_1* Carrier		% Dose in urine			% Labeled iodine in urine as T_1*			% Labeled iodine in urine as iodide		
No.	Age	Sex		Form	T_1 (μg.)	1st hr.	2nd hr.	3rd & 4th hr.	1st hr.	2nd hr.	3rd & 4th hr.	1st hr.	2nd hr.	3rd & 4th hr.
1A	37	F	Obesity	*dl*	176	22.4	14.8	13.4	39.3	29.9	16.7	33.0	37.5	48.6
1B				*l*	205	8.0	5.4	11.4	2.8	2.2	.8	77.2	77.4	82.3
2A	59	M	Undetermined process, rt. lung	*dl*	176	26.7	16.1	14.7	33.3	19.3	14.2	39.9	57.2	58.7
2B				*l*	205	10.8	8.3	12.1	1.9	1	†	86.4	93.0	†
3	17	M	Diabetes	*dl*	176	25.7	11.8	12.8	32.9	20.6	11.8	32.3	48.4	56.6

† Specimen lost.

The findings are shown in Table 3 and are illustrated in Figure 3. In the table no correction has been made for paper smearing. The values are given as percentages of the total number of counts recovered from the chromatogram. The metabolism of *l*-T_1* differed from that of *dl*-T_1*. The urinary excretion rate of labeled iodine was faster after administration of the racemic *dl*-preparation than after *l*-T_1*.

After administration of *l*-T_1* the labeled iodine of the urine was found

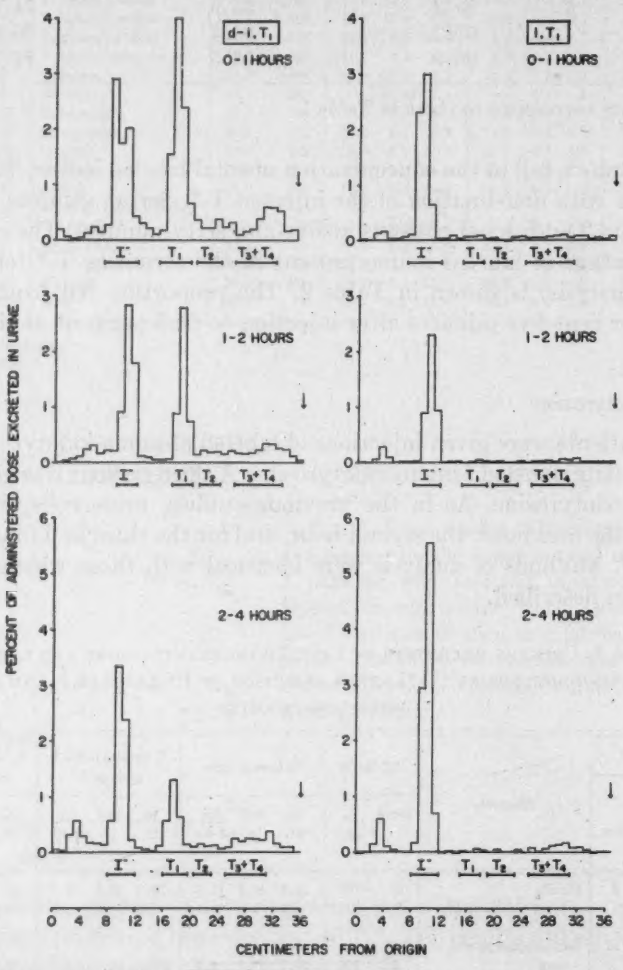

FIG. 3. Chromatograms obtained on urine specimens of an obese patient after intravenous administration of I^{131}-labeled mono-iodotyrosine (T_1*), first in the *dl* form and later in the *l* form. The construction of the charts is the same as in Figure 1. A butanol-acetic acid solvent system was used.

almost entirely in the iodide zone. There was a small peak between the origin and the iodide zone. In contrast, after injection of *dl*-T_1* a large fraction of the labeled iodine in the urine appeared in the T_1 zone. In addition, there was a minor zone of labeled iodine between the origin and the iodide zone, and another near the solvent front.

DISCUSSION

The results following injection of labeled di-iodotyrosine were in all respects similar to those obtained by Albert and Keating (13) in 3 patients with treated myxedema. Their method of isolation of T_2 was by extraction. They also found that the excretion of T_2* was vanishingly small after four hours, and that there was an increase in the labeled iodide in the serum. Evidently, in the normal individual di-iodotyrosine is almost completely deiodinated; only a small fraction of the administered compound appears unchanged in the urine.

One patient who had severe diabetes mellitus excreted undeiodinated T_2* at a rate approximately twice that of any other patient in the series. This phenomenon might have been related to the large amount of glucose being resorbed by the renal tubules of this patient.

Three patients were goitrous (Table 1, #8, #13, and #14). One (#14) was the daughter of another (#13). In none was the excretory rate of T_2* larger than the average. These 3 patients had lived in areas where goiter is known to be mildly endemic.

Administration of large amounts of iodide to 2 patients (#2 and #12) made no noteworthy difference in the rate of appearance of T_2* in the urine. If exchange were the reason for the disappearance of T_2* from the serum and urine, then this process might have been accelerated by the enormous doses of potassium iodide relative to the usual daily intake. Actually, premedication with potassium iodide appeared to make no difference in the metabolism of T_2*.

Albert and Keating (13) suggested that deiodination of T_2 takes place in the various organs of the body. The high rate of disposal of T_2* estimated from their curves of serum concentration and urinary excretion was considered to be most consistent with an extrarenal deiodination process. Their estimates of renal clearance, however, were not so high as those known for certain other iodinated substances, such as Diodrast. Tissue slice experiments (21–23) have confirmed that many tissues can deiodinate di-iodotyrosine. Neither our data nor those of Albert and Keating exclude the possibility that a major site of deiodination is the kidney itself. The increase of labeled iodide in the serum after injection of *dl*-T_2* could be due as well to deiodination elsewhere as in part to leakage of iodide back into the blood after renal deiodination. Especially in the first hour of our experiments, amounts of labeled iodide which were large relative to T_2*

were appearing in the urine when the concentration of labeled iodide relative to T_2* in the serum was small. This can be explained by assuming either that the renal disposal rate of iodide was larger than that of T_2, or that T_2 was deiodinated in the process of renal clearance. Albert and Keating calculated the renal clearance of iodide to be in excess of that of T_2*. Their calculations were made from the second to the sixth hour after injection of T_2*. By the second hour the labeled iodine in the serum is predominantly in the form of iodide, and calculations accordingly would reflect largely the clearance of iodide, even if renal deiodination of T_2* were contributing to the labeled iodide in the urine. Estimations of renal clearance from their data for the first two hours show values for iodide clearance which are in excess of those usually observed. This could occur if the kidney directly excreted iodide which derived from deiodination of di-iodotyrosine in the kidney. Both the data presented here and those of Albert and Keating are consistent, therefore, with the hypothesis that deiodination of T_2* takes place during the process of renal clearance of this substance.

Immediately after injection of *dl*-T_2* the ratio of T_2* to labeled iodide in serum changed from that of the injection solution as the two components of the injected solution entered their own volumes of distribution and as the process of deiodination commenced (Table 2). At the end of an hour, approximately a third of the labeled iodine of the serum was in the form of inorganic iodide. The concentration of labeled iodide rose during the first hour, indicating that deiodination of the T_2* was proceeding faster than iodide was being removed from the serum.

The differences observed between the metabolic fate of *l*-T_1 and *dl*-T_1 were due to differences in the disposal of the *d* and the *l* forms. The findings indicate not only that labeled iodine appears in the urine more rapidly after administration of *d*-T_1, but also that a large part, if not all of the *d*-T_1 is excreted either undeiodinated or as degradation or conjugation products of T_1. The findings can be explained at least in part by assuming that deiodination of the *d* form takes place more slowy, or not at all.

Deiodination of *l*-T_1 proceeded so rapidly that conjugation or degradation products scarcely appeared in detectable amounts in the urine. A small amount of labeled iodine was found near the origin of the chromatograms. After administration of *dl*-T_1, significant zones of labeled iodine appeared near the origin and also near the solvent front. The labeled substances in these zones have not been identified.

SUMMARY

1. The fate of labeled *dl*-di-iodotyrosine has been observed in 16 studies on 15 subjects. One patient with diabetes mellitus was studied on two occasions.

2. All subjects excreted less than 8 per cent of the administered di-iodotyrosine as di-iodotyrosine in the first four hours after injection. The fraction of the dose appearing in the urine as di-iodotyrosine became vanishingly small after four hours. Most of the administered dose was excreted as iodide. The diabetic subject excreted unchanged almost twice as much of the administered di-iodotyrosine as any other patient. No labeled substances other than di-iodotyrosine and iodide were found in serum or urine.

3. Premedication with large doses of iodide, or varying the amount of carrier di-iodotyrosine over a range from 78 to 1,000 μg. appeared to make little, if any, difference in the results.

4. *dl*-Mono-iodotyrosine was given to 3 subjects, and *l*-mono-iodotyrosine was given to 2 of these. The *dl* form was excreted more rapidly than the *l* form.

5. *l*-Mono-iodotyrosine was excreted almost entirely as iodide, whereas in the first hour after the injection of *dl*-mono-iodotyrosine almost equal amounts of iodide and mono-iodotyrosine appeared in the urine. During later collection periods, the proportion of iodide in the urine rose and that of mono-iodotyrosine fell.

6. After injection of labeled mono-iodotyrosine, zones of unidentified iodinated substances were found in chromatograms of urine.

Addendum

Since the present report was accepted for publication, we have observed that the metabolism of *l*-T_2 appears to be indistinguishable from that of *dl*-T_2. Two patients with myxedema (supporting laboratory data) readily deiodinated *l*-T_2*.

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AN EVALUATION OF THE PROPHYLACTIC USE OF CORTISONE FOR PITUITARY OPERATIONS*

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BOTH the role of the anterior pituitary in the preservation of the body's homeostasis through its tropic hormones, and the derangements resulting from anterior pituitary insufficiency have been studied intensively. In acute anterior pituitary insufficiency the major therapeutic effort is directed toward correction of the effects of the resultant acute adrenocortical failure; under these circumstances, the effectiveness of cortisone is well established. This prompted the prophylactic use of cortisone, by ourselves and others, for patients undergoing operations on or about the pituitary gland. In this paper is presented a study of a large series of such cases, undertaken in order to determine the incidence of postoperative endocrine complications and the relationship of such complications to the preoperative use of cortisone and the preoperative presence of endocrine insufficiency.

CLINICAL MATERIAL AND METHODS

The case records of all patients who had craniotomies or operative procedures on or about the pituitary gland at the Mayo Clinic for the five years 1949 through 1953 were reviewed. One hundred and sixty-three such operations were performed on 153 patients. The yearly incidences were 27, 28, 30, 33 and 45 operations respectively. The patients' ages at the time of operation ranged from 4 to 69 years, with approximately half between 30 and 55 years. There were 75 males and 78 females. The pathologic diagnoses are listed in Table 1.

Cortisone or adrenal extract was not given before 60 operations; cortisone was given before 93 operations; and Kendall's adrenal cortical extract was given before 10. The cortisone was administered in a standardized manner in all but a few instances. Usually intramuscular injections of 200 mg. each were made forty-eight and twenty-four hours before operation, and on the morning of operation; these doses were followed by decreasing doses of cortisone during the next three to five (rarely ten) postoperative days. Kendall's adrenal cortical extract was given preoperatively in dosages of 40 to 60 cc. per

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TABLE 1. PATHOLOGIC DIAGNOSIS

Pathologic diagnosis	Operations	
Pituitary adenoma	127	
Chromophobe		89
Atypical mixed		20
Eosinophilic		7
Unclassified		11
Craniopharyngioma	25	
Cystic pituitary tumor	2	
Caseous granuloma of pituitary	1	
Metastatic adenocarcinoma to the pituitary	1	
Teratoma of pituitary	1	
Epidermoid cyst of pituitary	1	
Metastatic pinealoma to the pituitary	1	
Fibrous tissue replacing pituitary	1	
Infarcted pituitary tissue	2	
"Tumor" of pituitary	1	
Total	*163*	

day intramuscularly or intravenously; such treatment was started the day before operation and continued during the immediate postoperative period. Data concerning patients who received Kendall's adrenal cortical extract are included for completeness, but the small number of patients so treated makes comparisons with the other two categories difficult.

ANTERIOR PITUITARY INSUFFICIENCY

The degree of anterior pituitary insufficiency present before the 163 operations was classified. No anterior pituitary insufficiency was present before 97 operations; included in this category were not only the patients with no endocrine abnormality in the history or on physical examination or laboratory tests, but also 1) those who presented isolated symptoms, such as a slight to moderate decrease in libido or potentia, asthenia or cessation of menses after the age of 35 years, and 2) those who were found on physical examination to have such isolated conditions as minimal changes in skin, hair, testes or prostate. The chief laboratory criterion for inclusion in this group was urinary excretion of more than 3.0 mg. of 17-ketosteroids per twenty-four hours by the adult male and more than 2.0 mg. per twenty-four hours by the adult female. Levels lower than the customary lower limits of normal for the method of assay employed (6 mg., and 4 mg. for men and women respectively) were selected, because usually only a single determination was available. Values for urinary corticosteroids were not used as a primary qualification for inclusion in this

category, but only 1 patient[1] had less than the normal of 0.3 mg. per twenty-four hours as determined by the method of Mason for urinary formaldehydogenic corticosteroids. Low basal metabolic rates did not exclude patients from the "no insufficiency" group unless these low rates were accompanied by the clinical picture of impaired thyroid function or confirmatory laboratory tests. The distribution of the basal metabolic rates in this group is indicated in Table 2. The rates were determined before

TABLE 2. PREOPERATIVE BASAL METABOLIC RATES

BMR (%)	Pituitary insufficiency		
	None	Mild*	Marked*
>+36†	2	0	0
+35 to +26†	1	0	0
+25 to +16†	1	0	0
+15 to +6	0	1	0
+5 to −5	14	7	1
−6 to −15	26	17	5
−16 to −20	12	8	3
−21 to −25	9	2	0
−26 to −30	5	0	1
−31 to −35	0	1	1
Total	*70*	*36*	*11*

* No hypothyroidism by other criteria.

† Patients with BMR of more than +15 per cent had acromegaly and eosinophilic adenoma.

70 operations on patients with no pituitary insufficiency; 26 (37 per cent) of the rates were less than −15 per cent.

Mild anterior pituitary insufficiency was considered to be present at the time of 41 operations because of one or more of the following findings: 1) cessation of menstrual periods before the age of 35 years, usually without hot flushes and with decreased urinary concentrations of gonadotropin in patients undergoing 15 operations; 2) a 24-hour urinary excretion of 17-ketosteroids of less than 3.0 mg. by men and 2.0 mg. by women, usually associated with clinical evidence of mild pituitary insufficiency in patients undergoing 19 operations; 3) a diagnosis of hypothyroidism established on the basis of multiple tests, the clinical picture and, in a few instances, previous response to thyroid therapy in patients undergoing 5 operations

[1] This patient had a normal endocrine history, normal results of physical examination, and a urinary excretion of 17-ketosteroids of 5.1 mg. per twenty-four hours.

(none of these patients had the full-blown picture of pituitary myxedema at the time of operation), and 4) the clinical picture of mild pituitary insufficiency at the time of 3 operations, despite the absence of the preceding three characteristics. In this entire group the 24-hour urinary excretion of corticosteroids ranged from 0.22 to 0.88 mg., with 5 of 21 determinations showing less than 0.30 mg. The basal metabolic rates (excluding the 5 hypothyroid patients) are indicated in Table 2.

Marked anterior pituitary insufficiency was readily recognized clinically at the time of 25 operations. All patients in this category were adults and had unequivocally low values for 24-hour urinary 17-ketosteroids (less than 3.0 mg. for men and 2.0 mg. for women). Twelve had impaired thyroid function of varying degrees. Urinary excretion of corticosteroids ranged from 0.19 to 0.60 mg. per twenty-four hours, with 3 of 16 determinations yielding values of less than 0.30 mg. The basal metabolic rates of 11 patients, otherwise without apparent thyroid deficiency, are indicated in Table 2.

It should be emphasized that the nature of the material studied necessitated arbitrary criteria for the classification of the degree of anterior pituitary insufficiency. That this was not completely satisfactory is suggested by the tendency to hypometabolism evident in each of the three categories indicated in Table 2, despite the absence of other signs of hypothyroidism. None of the patients had malnutrition as a possible cause of hypometabolism. It might have been preferable to consider all cases with basal metabolic rates of less than −15 per cent as cases of hypothyroidism, despite the absence of other evidence. If this criterion were used, it would perhaps be better to refer to the group with " no preoperative pituitary insufficiency" as having "no or minimal" insufficiency. If the classification were altered so that a basal metabolic rate of less than −15 per cent moved the patient from the group with no insufficiency to the group with mild insufficiency, the correlation to be outlined would not be changed significantly.

ENDOCRINE COMPLICATIONS

Our interest was directed especially to the complications which could be ascribed to anterior pituitary failure, either existing preoperatively or resulting from operation on the pituitary. Long-term effects were excluded by considering only complications beginning within five days after operation. Diabetes insipidus from posterior pituitary failure was tabulated separately and not included as an "endocrine complication" for the purpose of this study. No endocrine difficulties appeared during the operation itself; 1 patient had hypotension during operation but this appeared related to excessive bleeding and the blood pressure responded to transfusion.

Endocrine complications developed after 10 of the 163 operations. In this

retrospective analysis it was necessary to rely on the recorded evaluation of the metabolic consultant caring for the patient, the response to specific therapy, and the presence of other more likely explanations for a patient's difficulty. Only 1 patient was considered to have the usual picture of acute adrenal insufficiency with fall in blood pressure, fever, vomiting and diarrhea after operation for a chromophobe adenoma. She responded to treatment with adrenal cortical extract, despite the presence of marked anterior pituitary insufficiency. Four months later she had another operation on the pituitary. Despite preoperative administration of desoxycorticosterone acetate, thyroid and conjugated estrogenic substances, fever and hypotension again developed after operation. The patient responded somewhat to adrenal cortical extract but died on the fourth postoperative day. Hypotension, often accompanied by fever, lethargy or vomiting, developed after 5 other operations. Because of the response to treatment with cortisone, hydrocortisone or adrenal cortical extract, these patients were also considered to have endocrine complications. Stupor following operation on the pituitary and responding to cortisone has been reported (1). After 3 operations in our series, marked drowsiness or stupor without hypotension developed. The condition responded in each instance to cortisone or adrenal cortical extract and is considered an endocrine complication.

No other definite endocrine complication could be found. Although it is possible that preoperative myxedema might alter the outcome of operation, we were not able to discover any constant pattern. Thus, our 10 endocrine complications were apparently directly related to adrenocortical failure. Complications considered as being nonendocrine included fever, confusion, neurologic changes, hypertension, pulmonary embolism and the like. In a few instances there was drowsiness or hypotension but the absence of a clear-cut response to treatment with cortisone or adrenal cortical extract, and the over-all picture, led to their inclusion in the nonendocrine group. Although not considered as endocrine complications for this report, it should not be inferred that the metabolic consequences of postoperative diabetes insipidus are minor. Several patients had protracted water and electrolyte difficulties because of this condition. Frequently confirmed in this series was the relationship of diabetes insipidus to the competency of anterior pituitary function and the use of hormone replacement therapy. For example, treatment of panhypopituitarism with cortisone occasionally resulted in the clinical manifestations of diabetes insipidus, whereas the latter appeared to have been either minimal or absent before such treatment. Of interest is the observation that diabetes insipidus developed after about 20 per cent of the operations on patients who did not have postoperative endocrine complications, whereas no diabetes insipidus was noted after the 10 operations which were followed by endocrine complications.

The chief purpose of this study was to determine what circumstances

appear to be related to the development of endocrine complications, as here defined, after operations on the pituitary. No correlation was noted with such data as probable duration of lesion, abnormality of the visual fields, nature of change in the sella turcica on roentgen-ray examination, previous roentgen therapy or operation on the pituitary, pathology of the pituitary lesion, and the postoperative development of chronic panhypopituitarism. Of significance were the preoperative treatment with cortisone and the degree of anterior pituitary insufficiency present before operation. The relationship of these two factors to the incidence of postoperative complications is shown in Table 3.

When the patients treated preoperatively with adrenal cortical extract are omitted, no pituitary insufficiency was present at the time of 94 operations and postoperative endocrine complications developed after only 4 of these operations. This should be compared to development of such complications after 5 of the 22 operations in cases of marked preoperative pituitary insufficiency. The difference (Table 3) is significant ($p < .02$). This indicates that patients with marked anterior pituitary insufficiency undergoing pituitary operations are more likely to have postoperative endocrine complications than those with no preoperative insufficiency.

Endocrine complications developed after all 3 operations on patients who had no preoperative therapy for marked pituitary insufficiency. They developed after only 2 of 19 operations on patients with marked insufficiency who received cortisone preoperatively. Only in this group with marked insufficiency was there any statistical indication that preoperative

TABLE 3. RELATIONSHIP OF PREOPERATIVE TREATMENT AND PREOPERATIVE PITUITARY FUNCTION TO POSTOPERATIVE ENDOCRINE COMPLICATIONS

Preoperative therapy	Postoperative endocrine complication	Preoperative pituitary insufficiency			Total
		None	Mild	Marked	
None	No	45	9	0	54
	Yes	3	0	3	6
Cortisone	No	45	27	17	89
	Yes	1	1	2	4
Adrenal cortical extract	No	3	4	3	10
	Yes	0	0	0	0
Total		*97*	*41*	*25*	*163*

administration of cortisone reduced the incidence of postoperative endocrine complications ($p < .01$).

In view of these correlations, plus the fact that the ultimate development of panhypopituitarism did not appear to be related to postoperative endocrine complications, it is possible that these complications may really be independent of the exact nature of the surgical procedure (whether or not on the pituitary) and may be due only to the effect of severe stress on preexisting adrenal insufficiency. The development of endocrine complications in those patients classified by us as having no or minimal pituitary insufficiency before operation would then merely reflect our inability to anticipate the true functional capacity of the adrenal cortex during stress and to recognize changes in this capacity with more subtle deficiencies of pituitary function. In any event, cortisone appears to be indicated preoperatively for such patients.

MORTALITY

There were 19 hospital deaths in this series—an over-all mortality, on the basis of operations, of 12 per cent. Only 1 of these deaths (described in the previous section) was attributed to an endocrine complication. Representative causes of death of the other 18 patients included infarction of the brain, extradural hematoma, herniation of the cerebellum through the foramen magnum, pulmonary embolus, cerebral edema, and increased intracranial pressure. Although in several instances the cause of death was not as clear, a critical review did not appear to warrant ascribing the major role to endocrine failure. The relationship of a fatal outcome to preoperative therapy and the degree of preoperative anterior pituitary insufficiency is shown in Table 4. After operations on patients receiving cortisone prophylactically, the mortality rate was 6 per cent compared to 17 per cent for those receiving no preoperative therapy. Other things being equal, this suggests that cortisone reduces the mortality after operations on the pituitary gland. This could be by specific replacement in cases of adrenocortical failure, or through some other mode of action. Admittedly the criteria for the diagnosis of an endocrine death in the face of severe neurologic disturbance are not wholly satisfactory, but only 1 death in our series was considered as primarily endocrine. In addition, in contrast to the incidence of endocrine complications, the mortality rate was not significantly different for each of the three degrees of preoperative anterior pituitary function (whether taken collectively or for each preoperative therapy separately). Thus, on the basis of the available data, the improved mortality figures when cortisone was given preoperatively are not completely explained on the basis of specific replacement therapy.

The figures for postoperative endocrine complications and deaths in those patients receiving adrenal cortical extract preoperatively are in-

TABLE 4. HOSPITAL DEATHS AFTER OPERATIONS ON PITUITARY GLAND

Preoperative therapy		Preoperative pituitary insufficiency			Total operations
		None	Mild	Marked	
None	Total	48	9	3	60
	Deaths	8	1	1	10
Cortisone	Total	46	28	19	93
	Deaths	2	3	1	6
Adrenal cortical extract	Total	3	4	3	10
	Deaths	1	1	1	3
Total	*No.*	*97*	*41*	*25*	*163*
	Deaths	*11*	*5*	*3*	*19*

cluded in Tables 3 and 4, but the small number of cases makes interpretation difficult. These patients were therefore not included in the preceding correlations.

The general impression among the members of the neurosurgical service of the Mayo Clinic is that administration of cortisone has not resulted in any operative difficulty or postoperative morbidity (as for example, cerebral edema) in pituitary operations.

SUMMARY

A review of the case records of the 153 patients who had 163 operations on the pituitary revealed that postoperative endocrine complications were significantly more frequent in patients who had marked anterior pituitary insufficiency before operation. In this latter group, cortisone given preoperatively was decidedly effective in reducing the incidence of postoperative endocrine complications. It is possible that these endocrine complications are due to severe stress on pre-existing adrenal insufficiency and are independent of the exact nature of the operation. The mortality rate appeared unrelated to endocrine derangement preoperatively or postoperatively; yet the mortality rate was lower in the group treated with cortisone.

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CHROMATOPHOROTROPIC ACTIVITY OF HUMAN BLOOD: REVIEW OF 1,200 CASES

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SINCE 1923 sporadic reports have appeared on the presence of chromatophorotropic hormone or melanocyte-stimulating hormone in mammalian blood (1–20). Unfortunately, many of these studies were performed with inadequate techniques and they were, moreover, wrongly interpreted and poorly understood because their relation to the adrenal gland was then still unknown.

The advent of the adrenocorticotropic (ACTH) era brought forth new studies on this subject with improved techniques and test objects, especially those by Johnsson and Högberg (21,22) and Sulman and collaborators (23–33). The findings of both groups, who worked independently, were confirmed by many other observers (34–60). Several of the aforementioned authors (22, 47, 59, 60), as well as Holzbauer (61), Carstam (62) and Halkier (63) also dealt with the assay of chromatophorotropic hormone in the blood. Most of them studied only a very limited number of cases. The reason for such superficial study is obvious: the technical difficulties involved did not allow the use of the present methods for routine purposes.

We have tried to overcome this difficulty by developing a simple routine method, which has enabled us to study until now 1,200 clinical cases in addition to 800 normal cases and a large number of selected psychiatric cases on which we shall report separately.

TECHNIQUE

Blood samples were drawn from the cubital veins with a minimum of stress. Usually a 20-ml. sample was taken for an assay, but should this prove difficult, 10 ml. sufficed. It is important that blood kathepsins be immediately destroyed before they inactivate the chromatophorotropic hormone (*see* subsequent sections). Thereafter the specimen can be kept for at least one week at room temperature without loss of potency.

The hormone assay was carried out by the method described by us in 1952 (26). As we have slightly modified the method for routine purposes, its present form is as follows:

Concentration of chromatophorotropic hormone from blood

1. A 10-ml. or 20-ml. sample of blood withdrawn from the cubital vein is immediately transferred to a bottle containing 20 ml. of distilled acetone and 0.1 ml. of glacial acetic

acid. This procedure inactivates the hormone-decomposing enzymes of the blood and precipitates inert proteins.

2. The suspension is sucked through a 1–2 inch Buchner funnel with Whatman filter-paper No. 1, and the precipitate is washed with 5 ml. of 50 per cent acetone. The pooled filtrates (approximately 25 ml.) contain the whole chromatophorotropic hormone (full recovery).

3. This filtrate is poured into a 100-ml. bottle and 60 ml. of distilled acetone is added, which precipitates the hormone within an hour. In order to facilitate precipitation and sedimentation, 10 ml. of ether is added immediately and the bottle is allowed to stand overnight in the refrigerator.

4. Next morning the supernatant fluid is decanted; the precipitate is washed with about 5 ml. of ether, the ether decanted, and the residue freed from traces of ether by placing the bottle upside-down for about thirty minutes.

5. The hormone precipitate is dissolved quantitatively in 0.8 ml. of N/20 NaOH with the addition of 1–2 drops of a standard bromthymol-blue indicator solution. If the precipitate does not completely dissolve, the bottle should be shaken in a shaking apparatus. Finally, some drops of N/4 acetic are added until the solution becomes green, avoiding, however, isoelectric precipitation. In those rare cases in which an insoluble precipitate still remains, the soluble part only is used for injection.

6. The concentrated hormone solution (approximately 1 ml. equivalent to 20 ml. of blood) is injected into green-adapted tree frogs as follows:

Frog No. 1—0.1 ml. (*i.e.*, 2 ml. of blood) corresponding to 500 frog units per liter of blood.

Frog No. 2—0.3 ml. (*i.e.*, 6 ml. of blood) corresponding to 166 frog units per liter of blood.

Frog No. 3—0.6 ml. (*i.e.*, 12 ml. of blood) corresponding to 83 frog units per liter of blood.

For the routine method we prefer to inject first only frog No. 2 (0.3 ml.). If within ten minutes the reaction is negative, the remainder of the solution (0.7 ml.) is immediately injected into the same frog and thus the result is obtained with 1 ml. of solution (*i.e.*, 20 ml. of blood) corresponding to 50 frog units per liter blood. If within ten minutes the reaction is positive, frog No. 1 is injected and, according to the outcome after ten minutes, the dosage is either increased immediately in frog No. 1 by 0.1 or 0.2 ml., or the dosage is decreased, using one or more additional frogs for titration. This routine method permits the assay of about 40 to 50 blood samples in twelve to twenty-four hours.

Test animals

It is essential that only the tree frog be used for these tests. The tree frog (*Hyla arborea*) is a reliable, and cheap test animal which is easy to handle; it is available in many laboratories because of its use in pregnancy tests. When kept for one week in captivity on a white table, tree frogs assume a uniform bright green color (stage of adaptation). After one week the frogs are kept for one day on a brown table. If they remain green, they are ready for use (stage of challenge). The test itself is carried out on a white table in order to prevent "false positives." When injected with melanophore hormone (intermedin) or with corticotropin, the green-adapted frogs assume a dark olive color within ten to fifteen minutes and, after excessive doses, they become dark brown within two to four hours. They are injected with a 22–25 gauge needle which is introduced from the hindleg through the gluteus region into the dorsal lymphsac (or into

the ventral subcutis). This technique prevents leakages. Neutral pH of the fluids to be injected is essential, and it should be adjusted carefully in order to prevent toxic effects.

SPECIFICITY OF TEST

Specificity of the Hyla test

Nonspecific chromatophore reactions obtained in Rana and Xenopus have recently been reported. It seems that not only *Rana ridibunda* but also *Rana esculenta* (34–36, 64) and *Xenopus laevis* (37, 65) are not fully reliable test animals. Stolte and associates (66) even stated that the hypophysectomized *Rana esculenta* reacted to pregnancy gonadotropin and prolactin. On the other hand, Hyla has proved to be highly specific in experiments *in vivo* as well as *in vitro* (25, 67). For this reason earlier experiments on melanophore hormone activity and occurrence should be re-examined, the use of Hyla being an indispensable condition if specificity is required.

In a series of papers (23–25, 27) we have furthermore presented evidence that the Hyla test is specific and cannot be elicited by biogenic amines or amino acids, nor by the commonly available hormones.

Evaluation of the test

The result of the blood examinations is expressed in frog units. This unit is approximately 1 gamma of chromatophorotropic hormone; *e.g.*, 1 mg. (USP unit) of any preparation of corticotropin on the market contains at least 1,000 frog units of chromatophorotropic hormone (27). A normal person has always less than 50 frog units per liter of blood. The normal value lies at 10 frog units per liter of blood for males and females. A value of 50 frog units per liter is definitely pathologic. Highest values found were 2,000 frog units per liter; on the average we found increased values of 100–200 frog units of chromatophorotropic hormone per liter of blood in the various conditions studied by us. In Tables 1–6 we have designated our results as follows:

Negative—less than 50 frog units per liter of blood.
Positive—50 to 2,000 frog units per liter of blood.

RESULTS

For the sake of convenience, the different diseases studied have been divided into 6 arbitrary groups (Tables 1–6).

1. *Endocrine conditions* (Table 1)

In this group increased levels of chromatophorotropic hormone were found in the following conditions:

Hypoadrenalism (Nos. 1–3). In Addison's disease a positive finding was so regular that we introduced it as a standard method for diagnosing un-

treated cases. The reaction becomes negative (5 cases) within three days when the patient is treated with cortical steroids. The significance of this observation will be discussed later. It is not surprising that adrenalectomized patients also show positive reactions. Of interest are the schizophrenic patients who were treated with chlorpromazine. At the start of the treatment 17-ketosteroids disappeared from the urine. This transient phenomenon was accompanied by a steep increase of chromatophorotropic hormone in the blood, which subsided gradually. A detailed study of this phenomenon is now being conducted.

Hyperadrenalism (combined with hyperpituitarism) (Nos. 4–13). Cushing's

TABLE 1. CHROMATOPHOROTROPIC REACTION IN ENDOCRINE CONDITIONS

Disease	Negative	Positive	Disease	Negative	Positive
1. Addison's disease	5	25	14. Diabetes insipidus	1	0
2. Adrenalectomy	0	3	15. Chiari-Frommel syndrome	1	0
3. Hypothalamic depression by chlorpromazine	18	7	16. Hermaphrodism	2	0
4. Cushing's disease	0	3	17. Pseudohermaphrodism	2	0
5. Cushing's syndrome	30	15	18. Adiposity	5	0
6. Adrenal tumors	0	2	19. Gigantism	3	0
7. Pheochromocytoma	0	3	20. Nanosomia	2	0
8. Hirsutism	1	3	21. Hypothyroidism	2	0
9. Adrenogenital syndrome	4	2	22. Panhypopituitarism	11	0
10. Virilism	2	4	23. Froehlich syndrome	1	0
11. Cortisone psychosis	2	1	24. Laurence-Moon-Biedl syndrome	1	0
12. Hyperthyroidism	8	3	25. Infantilism and debility	3	0
13. Diabetes mellitus	10	3			

disease, when caused by a primary basophil tumor of the pituitary, has always been associated in our assays with greatly increased blood levels of chromatophorotropic hormone. This fact is not surprising and will be discussed. The patients with pituitary Cushing's disease exhibited a significant feature: in all of them the level of chromatophorotropic hormone was extremely high—250–500 frog units per liter of blood—and their complexion was rather dark.

These cases were well differentiated from the next group, *i.e.*, Cushing's syndrome, in which a primary adrenocortical tumor is present. About 50 per cent of these adrenal patients also had an increased level of chromatophorotropic hormone; however, it was never more than 100 frog units per liter. This difference allows a quantitative approach to the problem of differential diagnosis of Cushing's syndrome. According to our experience,

the quantitative results of the chromatophore test in Hyla may be tabulated as follows:

a) Cushing's disease (primary pituitary tumor)—250–500 frog units per liter of blood.
b) Cushing's syndrome (adrenal) with hyperplasia of the pituitary basophils—50–100 frog units per liter of blood.
c) Cushing's syndrome (adrenal) without involvement of the pituitary—negative reaction.

Thus there exists an intermediate type of adrenal Cushing's syndrome with hyperplasia of the anterior pituitary lobe characterized by an increase of the chromatophore hormone. In our series, probably there existed also a certain degree of hyperpituitarism in the cases of adrenal tumor, pheochromocytoma, hirsutism, adrenogenital syndrome, virilism, severe hyperthyroidism and diabetes mellitus which showed a positive reaction. In the last two conditions there was certainly no hyperadrenalism, but apparently only hyperpituitarism.

Miscellaneous endocrine conditions (Nos. 14–25). In the following endocrine conditions no increase of chromatophorotropic hormone could be detected: hermaphrodism, pseudohermaphrodism, adiposity, gigantism, nanosomia, hypothyroidism, panhypopituitarism, Froehlich syndrome, Laurence-Moon-Biedl syndrome, infantilism and debility of suspected endocrine origin.

2. *General medical conditions* (Table 2)

In this group were various medical conditions, some of which are known to be accompanied by a low level of 17-ketosteroids (17-KS) in the urine.

Conditions with low 17-KS excretion (Nos. 1–2). In rheumatoid arthritis the number of positive reactions was particularly high. Acute cases with an increased sedimentation rate always showed a positive reaction; in chronic cases they were not so frequent. This fact coincides well with the low level of 17-KS described by us for this disease (Cohen and Sulman (68)).

In cirrhosis of the liver also, there were increased levels of chromatophorotropic hormone in the blood. It should be borne in mind that in such disturbances of the liver there is also a low 17-KS excretion, the cause of which is still obscure.

Circulatory stress (Nos. 3–9). In this group of diseases an increased level of chromatophorotropic hormone was present only when the patients were in an acute stage of stress. The hormone appeared especially in recent myocardial infarction, acute heart failure, hypertensive crisis, status asthmaticus, acute pulmonary infarct, severe gastroenteritis, and typhoid

TABLE 2. CHROMATOPHOROTROPIC REACTION IN GENERAL MEDICAL CONDITIONS

Disease	Negative	Positive	Disease	Negative	Positive
1. Rheumatoid arthritis	75	30	15. Hematemesis	3	0
2. Cirrhosis of the liver	2	2	16. Hepatitis	4	0
3. Myocardial infarction	6	2	17. Homologous serum jaundice	3	0
4. Heart failure	3	8	18. Arthralgia	3	0
5. Hypertension	8	3	19. Glomerulonephritis	4	0
6. Asthma, bronchial	1	1	20. Status febrilis	5	0
7. Pulmonary infarct	2	2	21. Miliary tuberculosis	2	0
8. Gastroenteritis	4	1	22. Malnutrition and anorexia nervosa	4	0
9. Typhoid fever	3	6	23. Myotonia, congenital	2	0
10. Buerger's disease	1	0	24. Myasthenia gravis	3	0
11. Thrombophlebitis	3	0	25. Adynamia	3	0
12. Anemia	3	0			
13. Polycythemia vera	3	0			
14. Sprue	4	0			

fever. We had the definite impression that the rare cases of positive reactions observed in these acute conditions were only due to circulatory stress, since the reactions became negative when the acute circulatory condition subsided.

Miscellaneous conditions (Nos. 10–25). Other medical conditions studied yielded negative reactions, *e.g.*, Buerger's disease, thrombophlebitis, anemia, polycythemia vera, sprue, hematemesis, hepatitis (acute and chronic), homologous serum jaundice, arthralgia, glomerulonephritis, status febrilis of unclear origin, miliary tuberculosis, malnutrition, anorexia nervosa, myotonia congenita, myasthenia gravis, and adynamia of non-adrenal origin.

3. *Gynecologic and obstetrical conditions* (Table 3)

Pregnancy and allied conditions (Nos. 1–5). The level of chromatophorotropic hormone in the blood is often increased in pregnancy, but this is not the rule. According to Bromberg, Sadowsky and Sulman (32), up to 20 per cent of pregnant women show positive reactions with the frog test. It makes no difference in which month of pregnancy patients are examined. This conforms with observations that often, but not always, pregnant women show increased pigmentation at sites which are not target organs of the estrogenic hormones, *e.g.*, the abdomen. On the other hand, pigmentation of the nipples and vulva is without doubt an estrogenic effect. The investigations of Sulman and Bergmann (31) have shown that chromatophorotropic hormone is formed in the human placenta. Thus, in-

TABLE 3. CHROMATOPHOROTROPIC REACTION IN GYNECOLOGIC AND OBSTETRIC CONDITIONS

Disease	Negative	Positive	Disease	Negative	Positive
1. Pregnancy	136	27	13. Galactorrhea	3	2
2. Eclampsia	1	2	14. Habitual abortion	3	0
3. Toxemia of pregnancy	4	1	15. Ovarian cyst	3	0
4. Mole	2	3	16. Amenorrhea primaria	3	0
5. Puerperium	8	2	17. Oligomenorrhea	3	0
6. Delivery	46	14	18. Hypomenorrhea	4	0
7. Delivery by obstetric forceps	1	4	19. Menopause	4	0
8. Cesarean section	2	7	20. Myoma uteri	3	0
9. Abortion	6	1	21. Prolapsus uteri	4	0
10. Curettage	4	2	22. Sterility	3	0
11. Climacterium	4	6	23. Fetal blood	3	0
12. Metrorrhagia	3	2	24. Hydrops fetalis	3	0
			25. Asphyxia neonatorum	4	0

creased production of this hormone occurs in every case of pregnancy, but the blood level and its effect on the cutaneous pigmentation of the pregnant women varies from patient to patient.

Positive reactions were also found in eclampsia, toxemia, puerperium and mole, but not as a rule. Hence it appears that with regard to the chromatophorotropic hormone there is no principal difference between these conditions and pregnancy.

Obstetrical stress (Nos. 6–10). The percentage of positive reactions during and after delivery was higher than in pregnancy. It is obvious that this increase is connected with the stress of delivery. The same holds true for the positive reactions observed after delivery by obstetrical forceps, cesarean section, abortion and curettage. The percentage of positive reactions in this group will always depend upon the stress experienced by the individual patient.

Gynecologic disturbances (Nos. 11–22). Some severe hormonal disturbances were observed in this group and they were associated with an increased chromatophorotropic level—*e.g.*, climacterium, metrorrhagia, galactorrhea. On the other hand negative reactions were found in habitual abortion, ovarian cysts, primary amenhorrhea, oligomenorrhea, hypomenorrhea, menopause, myomata, prolapsus uteri and sterility.

Fetal pathology (Nos. 23–25). We had the opportunity of examining fetal blood. It was always free of chromatophorotropic hormone, even in cases of hydrops fetalis and asphyxia neonatorum.

4. *Dermatologic conditions* (Table 4)

The rare occurrence of chromatophorotropic hormone in this group is astonishing and proves the specificity of the test.

Conditions with increased chromatophorotropin level (Nos. 1–4). Keratodermia is in our experience often connected with an increased level of chromatophorotropic hormone in the blood, which disappears only after treatment with cortisone. This puzzling fact is now under investigation.

There were also positive reactions in cases of acanthosis nigricans, which is not surprising in view of the pigmentation of these patients and the involvement of the pituitary basophils as described for the juvenile type of this condition by Rothman (69).

TABLE 4. CHROMATOPHOROTROPIC REACTION IN DERMATOLOGIC CONDITIONS

Disease	Negative	Positive	Disease	Negative	Positive
1. Keratodermia	1	8	11. Vitiligo	5	0
2. Acanthosis nigricans	7	2	12. Toxidermia	2	0
3. Pityriasis versicolor	0	2	13. Eczema	4	0
4. Alopecia totalis	0	2	14. Pruritus	3	0
5. Alopecia areata	4	0	15. Allergic dermatoses	4	0
6. Ultraviolet dermatitis	3	0	16. Pemphigus	5	0
7. Xerodermia pigmentosum	3	0	17. Sclerodermia	3	0
8. Negroid skin	3	0	18. Mycosis generalisata	3	0
9. Pellagra	2	0	19. Tinea corporis	4	0
10. Chloasmata	3	0			

In pityriasis versicolor and alopecia totalis the reaction was positive, whereas in alopecia areata it was always negative.

Conditions with normal chromatophorotropin level (Nos. 5–19). Conditions of increased pigment production need not necessarily be connected with increased chromatophorotropin levels. This is shown by the negative reactions observed in ultraviolet dermatitis, xerodermia pigmentosum, negroid skin, pellagra, and chloasmata.

In vitiligo, toxidermia, eczema, pruritus, allergic dermatoses, pemphigus, sclerodermia, mycosis generalisata and tinea corporis the reactions were likewise negative.

5. *Neurologic and psychiatric conditions* (Table 5)

In this group, conditions of psychologic stress take the leading part.

Psychic stress (Nos. 1–5). In psychoneurosis, schizophrenia and cyclothymia, the percentage of positive reactions was rather high; in epilepsy, it was lower.

The percentage of positive reactors among examination candidates was rather high, a phenomenon which will be further studied by us with additional means of assaying adrenal stress and the effect of nerve stimulants. There exists a report dating from 1934 that excitement may be able to provoke exceptional and transient melanosis in human beings (Bonorino Udaondo and Gonalons (70)).

Operational stress (Nos. 6–10). Patients well prepared for pneumoencephalography did not show a positive reaction, but production of chromatophorotropic hormone became rather high after the intervention. The findings were similar when we examined the blood of 20 patients chosen at random in the surgical ward. They had a normal chromatophorotropin level before operation, but in some of them the reaction became positive during operation, and in even a greater number (33 per cent) it became positive one to twelve hours after operation.

TABLE 5. CHROMATOPHOROTROPIC REACTION IN NEUROLOGIC AND PSYCHIATRIC CONDITIONS

Disease	Negative	Positive	Disease	Negative	Positive
1. Psychoneurosis	6	4	10. Surgical stress—after operation	13	6
2. Schizophrenia	8	3	11. Hypoglycemic shock	10	5
3. Cyclothymia	4	4	12. Electro-shock	54	46
4. Epilepsy	16	2	13. Retinitis pigmentosa	1	3
5. Psychologic stress (examinations)	67	15	14. Glaucoma	8	1
6. Pneumoencephalography—before	10	0	15. Parkinsonism	4	0
7. Pneumoencephalography—after	5	5	16. Ischialgia	3	0
8. Surgical stress—before operation	20	0	17. Sydenham's chorea	3	0
9. Surgical stress—during operation	14	3	18. Morgagni's syndrome (Adams-Stokes)	2	0
			19. Premenstrual tension	3	0

Shock reactions (Nos. 11–12). Our psychiatric wards afforded ample opportunities for studying the effect of shocks on the level of blood chromatophorotropin. In hypoglycemic shock the percentage of positive reactions was rather high (50 per cent). One hundred cases of electro-shock were studied; in 2 apprehensive patients the reaction was positive before the shock was given; and in 44 it became positive in ten to one hundred minutes after the shock. These results and their evaluation will be published elsewhere.

Miscellaneous positive reactions (Nos. 13–14). We have observed 1 positive reaction in glaucoma and 2 in retinitis pigmentosa, but we feel that these conditions should be studied further before definite conclusions can be drawn (*cf.* also earlier authors (16, 18, 20, 47)).

Miscellaneous negative reactions (Nos. 15–19). Negative reactions were found in parkinsonism, ischialgia, chorea (Sydenham), Morgagni's form of the Adams-Stokes syndrome and premenstrual tension.

6. *Tumors and allied conditions* (Table 6)

We studied 65 cases of various tumors; only 4 of them had an increased level of chromatophorotropic hormone in the blood. Three of the positive

TABLE 6. CHROMATOPHOROTROPIC REACTION IN TUMORS AND ALLIED CONDITIONS

Disease	Negative	Positive	Disease	Negative	Positive
1. Myoma uteri	3	0	12. Pituitary tumor (eosinophilic)	9	1
2. Carcinoma uteri	2	0	13. Melanoma maligna	4	0
3. Chorionepithelioma	2	0	14. Carcinoma cutis	3	0
4. Carcinoma ovarii	3	1	15. Carcinoma cardiae	2	0
5. Carcinoma vulvae	3	1	16. Carcinoma ventriculi	2	0
6. Lymphosarcoma	4	0	17. Carcinoma laryngis	2	0
7. Hodgkin's disease	3	1	18. Adenoma thyreoideae	2	0
8. Chronic leukemia	3	0	19. Carcinoma thyreoideae	2	0
9. Reticulum-cell sarcoma	2	0	20. Carcinoma bronchi	2	0
10. Ewing sarcoma	2	0	21. Carcinoma prostatae	2	0
11. Carcinoma mammae	3	0	22. Sarcoma fasciae	1	0

cases were due to a febrile condition and irradiation. The fourth case was a pituitary tumor with increased intracranial pressure. Further details of this research have been published (Hochman, Ratzkowski and Sulman (71)).

DISCUSSION

a. *Conditions with increased chromatophorotropin production*

The preceding results allow a clear-cut demarcation of the conditions in which the level of chromatophorotropic hormone in human blood is increased—hypoadrenalism, disorders with low 17-KS excretion, Cushing's disease, pregnancy, circulatory stress, obstetrical stress, psychic stress, operational stress, shock reactions, retinitis pigmentosa and certain dermatologic conditions, mostly those connected with pigmentation.

There are some miscellaneous endocrine disturbances (*cf.* Table 3, Nos.

11–13) in which the chromatophorotropic hormone may be temporarily increased. In all of them, the pituitary-adrenal axis may be involved.

b. *Regulation and site of chromatophorotropin production*

Production of the chromatophorotropic hormone starts within a few days after removal of the adrenal gland (22, 30). As we found, it disappears from the blood just as quickly when treatment with desoxycorticosterone (acetate), cortisone (acetate) or hydrocortisone is started. This is in correlation with the fact that in Addisonian patients treated with desoxycorticosterone, cortisone or aldosterone, melanin also tends to disappear more or less quickly (72–75). Hence we come to the conclusion that the production of the chromatophorotropic hormone by the pituitary gland follows the "push and pull" principle which governs and regulates also the secretion of the other anterior pituitary hormones. This regulation is especially evident in the case of the gonadotropic hormones, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and luteotropic hormone (LTH), which are suppressed by the gonadal steroids (particularly estrogens and androgens). The fact that even the production of LTH may be checked, if only partly, by estrogens and androgens has contributed to the current opinion that LTH should be assigned a place in the complex of gonadotropic hormones along with FSH and LH. From similar considerations Klinefelter (76) has followed our conception (23, 24) and that of Johnsson and Högberg (21, 22) by stating that the melanosis of the Addisonian patient can only be due to overproduction of chromatophorotropic principle.

The validity of the "push and pull" principle for chromatophorotropin excretion invites the question—which part of the pituitary gland produces this hormone? The fact that it is produced by the intermediate lobe has been proved by the studies reported in 1932 by Zondek and Krohn (9), who designated it as intermedin. In most animals the posterior lobe is the main source of intermedin. In man the intermediate lobe is but poorly developed and belongs morphologically more to the anterior lobe than to the posterior lobe. Hence we must assume either that chromatophorotropin is produced by the remnants of the human intermediate lobe or by the basophils of the anterior lobe. There is considerable evidence to support the latter assumption, as discussed in the following paragraph.[1]

It has been established beyond doubt that the chromatophorotropic

[1] Recently the assumption was advanced that there are several chromatophorotropic hormones, separable by countercurrent and paper chromatographic procedures, and that some of them originate in the posterior lobe (Laurentian Hormone Conference, 1955). We have shown the existence of two different chromatophorotropic hormones in the anterior and posterior lobes of the pituitary in dogs (33).

hormone, as assayed by the frog test, follows exactly the pattern of reactions established for corticotropin assayed by the ascorbic-acid depletion test (88). Both hormones are increased in Addison's disease, Cushing's disease, pregnancy, and various conditions of stress. The output of both hormones is checked by the administration of aldosterone, desoxycorticosterone, cortisone or hydrocortisone. In Table 7 is presented a synopsis of the values obtained for both hormones in the blood by various authors. A perusal of this table will convince the reader that so striking a similarity

TABLE 7. SYNOPSIS OF ADRENOCORTICOTROPIN AND CHROMATOPHOROTROPIN LEVELS IN HUMAN BLOOD. A REVIEW OF LITERATURE UP TO 1955

(Increased +; Normal −; Variable ±)

Condition	Adrenocorticotropin (ascorbic-acid test)	Chromatophorotropin (frog test)
Addison's disease and adrenalectomy	Sydnor and Sayers (77) + Sydnor, Kelley, Raile, Ely and Sayers (78) + Parrott (79) +	Sulman (30) + Johnsson and Högberg (22) + Shisume and Lerner (47) + Tilling and Thomann (60) +
Cushing's syndrome	Bornstein and Trewhella (80) + Parrott (79) + Upson (81) ± Sydnor, Sayers, Brown and Tyler (82) − Gray and Parrott (83) + Paris, Upson, Sprague, Salassa and Albert (84) −	Johnsson and Högberg (22) + Sulman (Cushing's disease) (29, 88) + Sulman (Cushing's syndrome) (29, 88) ±
Adrenogenital syndrome	Sydnor, Kelley, Raile, Ely and Sayers (78) ± Paris, Upson, Sprague, Salassa and Albert (84) + Parrott (79) +	Sulman (88) ±
Circulatory stress	Bornstein and Trewhella (80) + Upson (81) + Sydnor, Kelley, Raile, Ely and Sayers (78) + Paris, Upson, Sprague, Salassa and Albert (84) +	Johnsson and Högberg (22) + Sulman (88) +
Rheumatoid arthritis	Ceresa and Reyneri (85) ± Sydnor, Kelley, Raile, Ely and Sayers (78) ±	Sulman (88) ±
Pregnancy and allied conditions	Parrott (79) + Upson (81) ± Granirer (86) ± Gray and Parrott (83) ± Paris, Upson, Sprague, Salassa and Albert (84) −	Carstam (62) + Bromberg, Sadowsky and Sulman (32) ± Sulman and Bergmann (31) ± Johnsson and Högberg (22) + Shisume and Lerner (47) + Tilling and Thomann (60) +
Diabetes mellitus (severe forms only)	Bornstein and Trewhella (80) + Parrott (79, 87) +	Tilling (and Knick) (59) + Tilling and Thomann (60) + Sulman (88) +
Operational stress and shock	Bornstein and Trewhella (80) + Paris, Upson, Sprague, Salassa and Albert (84) + Parrott (79, 87) +	Holzbauer (61) + Johnsson and Högberg (22) + Sulman (88) +

of reactions has never been found between different hormones of the pituitary, except in the case of tropins or hormones, which are now considered to form a group and to be closely related to each other.

c. *Groups or complexes of tropins*

There are some precedents to illustrate and justify the conception of hormone groups or complexes:

1. *Vasopressin and oxytocin.* Both appear in the same site and extract of the posterior lobe and are separable only by elaborate procedures. De Vigneaud and his associates have shown in a series of papers that the difference in action can be attributed to very small differences in structure of these compounds—vasopressin contains a guanidine group, whereas oxytocin has a methyl group; and vasopressin has a phenyl group, whereas oxytocin has an ethyl group.

2. *FSH and LH.* Both hormones (follicle-stimulating, and interstitial-cell-stimulating) appear in the same aqueous extract of the anterior pituitary lobe and are separable only by elaborate procedures. In cases of increased production, both are increased in blood and urine; and both are decreased when patients are treated with gonadal steroids, in accordance with the "push and pull" principle. They are now considered to be two different factors of one hormone complex.

3. *Miscellaneous hormone complexes.* There exist even more than these two examples but their status is not sufficiently clarified, *e.g.*, the relationship between mammotropin and lactotropin, or between thyrotropin and exophthalmotropin.

d. *The corticotropin (ACTH) complex*

It has been established in previous papers (21, 22, 24–26, 34–41, 48–51) that every preparation of corticotropin contains a large amount of chromatophorotropic hormone which is completely inseparable in certain species and hence cannot be considered a mere contamination. To this fact is now added the evidence that the production of chromatophorotropin and, moreover, the reduction of its level in the human blood, follow exactly the pattern of the production of corticotropin and its withdrawal (*cf.* Table 7). Therefore, we can no longer escape the conclusion that chromatophorotropin, even though it be a separate hormone or factor or tropin, belongs to the group of corticotropin factors.

Separation of the different factors of the corticotropin complex is possible, just as in the case of the gonadotropin and pituitrin complex, but this fact does not allow us to distinguish in principle between tropins which, in accord with their chemical properties, occurrence, production and withdrawal, follow one unified pattern.

Nature makes no distinction between tropin factors, as does the bio-

chemist. It seems moreover that the corticotropin complex is composed of a multitude of factors, possibly distinguishable by molecular weight and other physical properties. The existence of the following corticotropin factors seems now to be proved:

A. Ascorbic-acid depleting factor (Reinhardt and Li (89)).
B. Basic adrenal weight-increase factor (Stack-Dunne and Young (90); Young (91)).
C. Chromatophorotropic factor (Sulman, 25).

This conception, advanced by us in 1952, has been adopted by most of the European workers.

The position has been ably summarized by Long (92) at the Fifth Conference (Adrenal Cortex) of the Josiah Macy, Jr. Foundation, where all participants agreed: "We certainly do not seem to have solved the question as to whether ACTH is a single substance or a mixture of hormones. The only thing we can say is that certain preparations of ACTH are most certainly a mixture of hormones. Whether there has yet been prepared a substance that represents the real tropic hormones of the adrenal cortex is undecided."

Benfey, Saffran and Schally (93) have recently tried in vain to separate corticotropin from intermedin. They conclude that their result "does not rule out the possibility that ACTH is present as an impurity in the melanophore-expanding hormone preparation, but it also strongly suggests that melanophore-expanding hormone may possess some slight inherent adrenocorticotropic activity."

e. *Blood assays of corticotropin*

Sayers (94) in a recent editorial concluded: "Obviously, assay of ACTH in the blood of man has definite limitations. . . . The method is not sensitive enough to detect ACTH in the blood of normal subjects under optimal conditions, nor is it sensitive enough to detect consistently an elevation in ACTH titer subsequent to the application of a noxious stimulus. We must continue to seek ways to increase the sensitivity of the method, for there is obviously a great need for information on the activity of the human adenohypophysis at a given instant following application of various intensities of stimuli." Sayers' statement is no doubt correct, as long as his ascorbic-acid depleting test is used for the detection of ACTH. If now the chromatophore test is introduced, we have an ideal method for the detection of ACTH which enables us to carry out routine examinations within twelve to twenty-four hours. The clinical application of this test on a large scale is easily possible and allows a single man to perform 40–50 examinations in the course of a day.

This test can be easily complemented by examination of the urine for chromatophorotropic hormone. A suitable technique has been reported by Shizume and Lerner (47) and its results have been fully corroborated by us, using a simplified technique which allows large-scale examinations for clinical purposes.

SUMMARY

1. Quantitative assay procedures for determining the level of chromatophorotropic hormone in human blood are described.

2. By this method the hormone is extracted from 10–20 ml. of human blood and the concentration is gauged on intact green-adapted tree frogs (*Hyla arborea*).

3. The reaction has proved useful for routine clinical purposes and allows the assay of 40–50 blood samples by a single person within twelve to twenty-four hours.

4. The test appears to be specific for chromatophorotropic hormone. The common biogenic amines and amino acids, as well as hormones, did not induce the chromatophore reaction in Hyla. In comparison to tests with other frogs (Rana), the Hyla test proved to be superior due to its specificity and easy performance.

5. A normal person has a level of less than 50 frog units per liter of blood. There is no difference between normal males and females.

6. Increased levels of chromatophorotropic hormone in the blood are found in the following conditions: hypoadrenalism; low urinary 17-ketosteroids; hyperadrenalism associated with pituitary basophilism (Cushing's disease and some cases of Cushing's syndrome); various adrenal and other endocrine disorders associated with hyperpituitarism; acute circulatory stress; pregnancy and allied conditions (rare); obstetrical stress; a few gynecologic disturbances; some dermatologic conditions; psychic stress (low incidence); operational stress and shock; and a few ophthalmic conditions (*e.g.*, retinitis pigmentosa).

7. The quantitative examination of the blood in "Cushing cases" with this test may be helpful in establishing a differential diagnosis as to whether the condition is pituitary Cushing's disease or adrenal Cushing's syndrome. The following blood levels of chromatophorotropic hormone were found:

in pituitary Cushing's disease—250–500 frog units per liter

in adrenal Cushing's syndrome—the results were mostly negative, but the level was slightly increased (50–100 frog units per liter) when the adrenal lesion was combined with benign basophil hyperplasia of the anterior pituitary lobe.

8. Comparison of the foregoing results with those obtained by other

authors who examined the level of corticotropin in the blood in various diseases showed so striking a resemblance as to give rise to the conclusion that increased production of corticotropin and of chromatophorotropic hormone always run parallel.

9. Moreover, the chromatophorotropic hormone has a typical relation to the corticosteroids, which is based on the "push and pull" principle: corticosteroids promptly suppress the production of chromatophorotropin. The opposite function—stimulation of corticoid production by chromatophorotropic hormone—is, however, less developed.

10. On the basis of these facts it is postulated that corticotropin should be considered as a complex of similar hormonal factors acting on different target organs, a concept which is generally accepted today for the gonadotropins and the posterior pituitary hormones. It is proposed to designate the different constituents of corticotropin as:

A. Ascorbic-acid depleting factor.
B. Basic adrenal weight-increase factor.
C. Chromatophorotropic factor.

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Addition made during proof-reading

Further research has shown us that there exist at least two chromatophore hormones in the mammalian pituitary gland. The first is formed by the basophil cells of the anterior lobe, and is identical with our C factor of the corticotropin complex. The second is formed in the posterior lobe, and there may be a third identical with Zondek's intermedin (Sulman and Eviatar (33)).

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EFFECT OF INTRAVENOUS TESTOSTERONE ON NITROGEN BALANCE IN MAN

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INTRODUCTION

THE anabolic effect of testosterone and its analogues has been adequately demonstrated by many workers. More recently, a preparation of testosterone dissolved in a solution of 25 per cent human serum albumin for intravenous administration was studied in our laboratories (1). This preparation was administered intravenously to 4 subjects and appeared to stimulate a sustained anabolic response in the 2 who received more than 150 mg. of the hormone.

Since human serum albumin is an effective plasma expander and inasmuch as albumin solutions are used in many situations involving the development of a catabolic response in injured patients, it was decided to study the effect of intravenous administration of solutions of testosterone and human serum albumin on the negative nitrogen balance precipitated by surgical procedures. Before initiating this investigation, the effect of this preparation on the nitrogen balance of control subjects was studied. This report is concerned with the results of these observations.

MATERIALS AND METHODS

Twenty-three satisfactory nitrogen balance studies were performed on 18 individuals. In most instances the testosterone (240 mg. dissolved in serum albumin) was administered intravenously over about a thirty-minute period, but in 2 subjects the infusion was given over a 24-hour period by slow intravenous drip. In 2 subjects intramuscular testosterone propionate in oil was administered at the rate of 25 mg. per day.

The subjects were all male—either convalescent patients or hospital personnel.

The testosterone in albumin solution was prepared by dissolving 13.47 grams of sterile micro crystals of testosterone in 3.8 liters of 25 per cent human serum albumin. The material was then sterilized by filtration and put into 100-ml. vials. The testosterone content was checked by analysis and found to be 2.4 mg. per ml.

Because of our inability to confirm the expected anabolic effects with this solution, two studies were carried out in which testosterone solutions were used which had been prepared according to the method of West (1, 2). These solutions were prepared by adding 200 mg. of free testosterone to 100 ml. of 25 per cent human serum albumin in a round-bottom flask. One hundred milligrams of dihydrostreptomycin and 50,000 units

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of penicillin G were added. The flask and contents were then agitated at 37° C. overnight. The undissolved testosterone was removed by filtration and the clear solution was used.

In contradistinction to the previous studies, an equivalent amount of nitrogen was not eliminated from the diet to compensate for the nitrogen in the albumin solution, since it has been shown that the infused albumin would be metabolized over a period of weeks (3, 4).

The subjects were fed a weighed diet which was analyzed for nitrogen by a macro-Kjeldahl method. Urine and stools were collected for at least five days as a base-line period before the testosterone was given. When the nitrogen balance checked within 0.5 Gm. per day, the subject was given testosterone in the manner described and the study was continued for another five to seven days.

The patients were weighed daily and all urine collections were checked for completeness by creatinine determinations. The nitrogen content of the diets was analyzed at least three times during the course of the study.

RESULTS

In every instance in which the intravenous testosterone was administered over about a thirty-minute period, the nitrogen balance was not significantly affected. A typical study is shown in Figure 1.

Two individuals (J.D. and P.E.L.) whose nitrogen balances were not influenced by the testosterone administered intravenously over a brief period were given the same material by a slow intravenous infusion which lasted about twenty-four hours. In J.D., there was a positive nitrogen balance of about 2 Gm. per day for two days, and in P.E.L. there was an

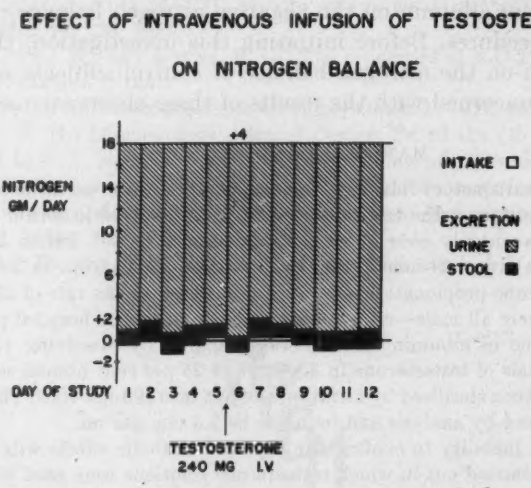

FIG. 1. Effect of infusion of 240 mg. of testosterone in 25 Gm. of human serum albumin infused over a thirty-minute period. The data are plotted according to the method of Moore and Ball (3). The albumin is not counted as intake.

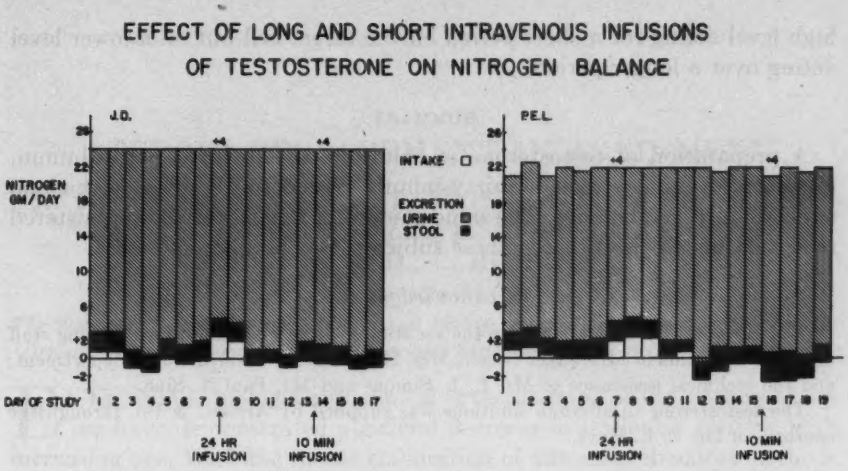

FIG. 2. Comparison of effects of infusion of 240 mg. of testosterone in 25 Gm. of human serum albumin infused over periods of twenty-four hours and ten minutes in Subject J.D. and twenty-four hours and thirty minutes in Subject P.E.L. In both cases the prolonged infusion resulted in a positive nitrogen balance; the short infusions had no effect.

even more marked positive balance for three days, with perhaps a compensating negative nitrogen balance after five days (Fig. 2).

Two subjects also received testosterone as testosterone propionate in oil intramuscularly, 25 mg. every other day. They exhibited the expected positive nitrogen balance, which appeared first on the second or third day after the beginning of therapy and reached a peak on the fourth or fifth day.

DISCUSSION

The failure to confirm the earlier studies (1) from our laboratories is disappointing. Since the earlier results were based only on 2 subjects and since we have had consistently negative findings in 18 subjects, it is felt that probably the earlier results were in part due to the elimination from this diet of the nitrogen equivalent to the albumin, and were in part fortuitous.

Although the large dose of testosterone (240 mg.) dissolved in human serum albumin was not anabolic when administered over a thirty-minute period, the same preparation was mildly anabolic when administered over a 24-hour period. This effect was, however, not equal to that of testosterone propionate given intramuscularly in smaller doses every day or two. This would suggest that the anabolic effect of testosterone depends not on a

high level acting for a short period on the target cell but on a lower level acting over a longer period.

SUMMARY

A preparation of testosterone in solutions of human serum albumin, given intravenously over a thirty-minute period to 18 subjects, had no effect on nitrogen balance. The same amount of this material, administered over a 24-hour period to 2 of these subjects, was mildly anabolic.

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STEROID REPLACEMENT IN AGED MEN*

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EXTENSIVE biologic and clinical investigations of the process of aging have demonstrated a general decrease in testicular activity with increasing age. With the recent elaboration of adequate chemical methods (primarily chromatographic techniques (1–6)) for the determination of androgenic and related steroids (C_{19}) or corticoids (C_{21} steroids) estimates of hormonal synthesis and release by the testis and adrenal have become possible. Particular application of chromatography, and especially paper chromatography, has been made to systematic studies of the changes in urinary 17-ketosteroid excretion with increasing age (7–10). On the basis of results from such studies and from experiments in which the recovery of orally administered steroids as individual urinary 17-ketosteroids was determined (11), approximate values have been calculated for the daily secretion of androgens and corticosteroids in men and women (12).

Investigations, designed to test the physiologic and psychologic action of steroid administration upon aged men, were undertaken in our laboratories. The nature and quantities of steroids administered were chosen so as to induce in these aged subjects blood concentrations of androgens and corticoids which would approximate those of young men in the age range of 20 to 40 years.

The effects of this steroid mixture were evaluated by determining the changes in the individual urinary 17-ketosteroids, blood and urinary inorganic ion concentrations, eosinophil counts, blood pressure, muscular endurance, neuromuscular coordination, memory, brain waves, blood pressure, and weight.

PROCEDURE

Subjects

The subjects for the study were 14 men aged 70–91 years, with a mean age of 81.2 years. They were all active and in fairly good health, with the usual sprinkling of con-

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ditions attendant upon the aging process, such as hypertension and arthritis. No patient with cardiac decompensation or with suspicious prostatic changes was used in this study.

Medication

For the first month of the investigation the men were given one placebo linguet[1] daily. Subsequently, a medicated linguet[1] was substituted, containing the following mixture of steroids: testosterone, 30 mg.; adrenosterone, 5 mg.; hydrocortisone acetate, 7 mg.; and corticosterone, 2 mg. This linguet was administered once daily for five months.

Methods

At monthly intervals, a 48-hour urine specimen was collected from each subject, and aliquots analyzed for creatinine (13), sodium and potassium (14), calcium (15), inorganic phosphate (16), total ketonic fraction (17) and individual 17-ketosteroids (11). One blood specimen was obtained from each subject during the premedication period and during the first and third months of therapy. Serum sodium levels and potassium (14), calcium (15) and inorganic phosphate (16) were determined.

Except for the psychologic tests and recordings of the brain waves, which were carried out at three-month intervals, all observations were made before and after the placebo period, then monthly during the period of medication, and also four and a half months after discontinuance. The muscular endurance test was suggested by the study of Simonson, Kearns and Enger (19); it consisted of lifting in each hand a five-pound dumbbell from the vertical position by the side, to shoulder height, twenty times a minute until fatigue was marked. This time was recorded. Although the end-point of marked fatigue was in part subjective, its validity could be observed objectively by the wider stance, the quivering of the wrists and the set of the facial muscles.

Neuromuscular coordination was tested by a modification of the Dunlap hand-steadiness test (20), in which the subject was given the task of keeping a pointer away from the sides of a hole. A series of holes of progressively smaller diameter was presented to the subject. Three trials lasting approximately eight and a half seconds each were allowed for each hole. When the subject reached the smallest hole or when he failed to attain a criterion previously established (a score of three seconds or better on two out of three trials) he was given ten trials on that hole. The number of errors and the length of the error-free time were recorded for each trial.

The psychologic tests (21) consisted of: 1) Wechsler-Bellevue digits (forward and backward); 2) Wechsler-Bellevue digit symbol; 3) Wechsler-Bellevue arithmetic; 4) Porteus mazes; 5) Wechsler memory scale (memory for story); 6) Stanford-Binet sentences; and 7) the Rorschach test. These procedures were used to indicate respectively: 1) level of attention and concentration; 2) effectiveness of learning; 3) arithmetical ability; 4) foresight and planning; 5 and 6) memory; and 7) psychologic developmental level (22).

Electroencephalograms were obtained on the subjects prior to medication and at the end of the medication period. They were analyzed according to the methods of Brazier (23) and Kaufman and Hoagland (24), which allow for a careful wave-by-wave determination of the various frequencies. Material so obtained can be corrected according to the percentage of time each frequency is present.

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[1] We are indebted to Drs. Elmer Alpert and Max Tishler of Merck & Co., Rahway, N. J., for supplies of placebo and steroid linguets.

TABLE 1. MEAN CONCENTRATION OF SODIUM, POTASSIUM, CALCIUM, INORGANIC PHOSPHATES AND EOSINOPHILS IN THE BLOOD OF AGED MEN DURING STEROIDAL THERAPY

Time blood sample obtained		Eosinophils (cells/cu.mm.)	Sodium (mEq./L)	Potassium (mEq./L)	Calcium (mg./100 ml.)	Inorganic phosphates (mg./100 ml.)
Before treatment		223 ± 82* [16]†	144 ± 1.7 [13]	4.82 ± 0.26 [13]	8.79 ± 0.29 [14]	2.80 ± 0.28 [16]
During treatment	1 mo.	234 ± 101 [15]	148 ± 5.6 [14]	5.39 ± 0.19 [13]	9.50 ± 0.33 [15]	2.72 ± 0.32 [15]
	3 mo.	240 ± 147 [14]	157 ± 6.0 [9]	4.79 ± 0.24 [14]	9.32 ± 0.31 [10]	2.84 ± 0.26 [14]

* Mean value ± standard error.
† Number of subjects.

RESULTS

Chemical changes in blood and urine (17-KS)

The mean concentration of inorganic ions and eosinophils in blood samples obtained from the groups of old men, prior to and during steroid administration, are presented in Table 1. In Table 2, the mean values for the excretion of urinary inorganic ions, creatinine and total 17-ketosteroids are listed. Except for urinary total 17-ketosteroids, no significant differences were observed between the mean excretions during the entire study.

Total 17-ketosteroid excretion increased during the first month of therapy (from 4.5 mg. to 17.1 mg. per twenty-four hours), remained constant during the remainder of therapy, and decreased to premedication levels (4.4 mg. per twenty-four hours) five months after cessation of therapy.

Variations in the excretion of individual 17-ketosteroids during the experimental period were noted. These are included in Table 3. Androstane-

TABLE 2. MEAN EXCRETION OF SODIUM, POTASSIUM, CALCIUM, INORGANIC PHOSPHATES, CREATININE AND TOTAL KETONIC 17-KETOSTEROIDS IN THE URINE OF AGED MEN DURING STEROIDAL THERAPY

Time urine sample obtained		Sodium (mg./hr.)	Potassium (mg./hr.)	Calcium (mg./hr.)	Inorganic phosphates (mg./hr.)	Creatinine (Gm./24 hr.)	Total 17-ketosteroids (mg./24 hrs.)
Before treatment (mean of 2 samples)		127.1 ± 39* [15]†	80.9 ± 19 [15]	4.22 ± 2.0 [15]	25.6 ± 5.7 [15]	0.92 ± 0.09 [11]	4.5 ± 0.33 [11]
During treatment	1 mo.	141.1 ± 51 [15]	91.8 ± 18 [15]	6.66 ± 2.7 [15]	25.2 ± 3.8 [15]	0.86 ± 0.10 [11]	17.1 ± 0.98 [11]
	3 mo.	110.7 ± 42 [13]	73.2 ± 24 [13]	5.33 ± 2.5 [13]	25.3 ± 6.6 [13]	1.03 ± 0.08 [11]	17.0 ± 1.01 [11]
	4 mo.	—	—	—	—	1.08 ± 0.08 [11]	19.2 ± 1.28 [11]
	5 mo.	—	—	—	—	1.11 ± 0.11 [11]	17.8 ± 0.82 [11]
After treatment (4.5 mos.)		116.0 ± 35 [14]	73.0 ± 22 [14]	5.00 ± 2.5 [14]	24.0 ± 6.0 [14]	0.92 ± 0.08 [11]	4.39 ± 0.39 [11]

* Mean value ± standard error.
† Number of subjects.

3α-ol-17-one (androsterone) excretion decreased continuously throughout the five months of steroid administration. The daily output of 3.37 mg. during the fifth month was significantly less than the initial treatment level of 6.81 mg. ($t=3.66$, $P<0.01$). In contrast, urinary etiocholane-3α-ol-17-one (etiocholanolone) remained constant through the fourth month of medication (average, 6.07 mg. per twenty-four hours), followed by a marked decrease (2.70 mg. per twenty-four hours for the fifth month; $t=4.03$, $P<0.01$). No significant changes in 11-oxygenated-17-ketosteroid excretion were evident.

TABLE 3. MEAN EXCRETION (MG./24 HRS.) OF SIX 17-KETOSTEROIDS IN URINE OF AGED MEN DURING STEROIDAL THERAPY

Steroid	Pre-therapy (mean of 2 readings) (11 subjects)	During treatment (mg./24 hrs.)			
		1 month (11 subjects)	3 Months (6 subjects)	4 Months (5 subjects)	5 Months (4 subjects)
Total 17-KS	4.5 ±0.33*	17.1 ±0.98	17.2 ±1.57	16.0 ±1.81	13.7 ±1.34
Androsterone	1.28±0.08	6.81±0.85	5.77±1.23	4.75±0.75	3.37±0.43
Etiocholane-3α-ol-17-one	1.20±0.12	5.63±0.43	6.33±1.15	6.25±0.80	2.70±0.37
Androstane-3α,11β-diol-17-one	0.25±0.06	1.12±0.25	1.51±0.36	1.08±0.07	0.81±0.17
Androstane-3α-ol-11,17-dione	0.13±0.02	0.23±0.02	0.22±0.04	0.28±0.08	0.15±0.02
11-Oxy-androsterone**	0.37±0.06	1.35±0.24	1.74±0.40	1.36±0.11	0.96±0.18
Etiocholane-3α,11β-diol-17-one	0.10±0.03	0.20±0.02	0.21±0.02	0.22±0.03	0.16±0.02
Etiocholane-3α-ol-11,17,dione	0.39±0.12	0.82±0.14	0.76±0.11	0.88±0.10	0.62±0.07
11-Oxy-etiocholanolone**	0.50±0.08	1.03±0.15	0.93±0.13	1.10±0.16	0.78±0.08

* Mean value ± standard error.

** 11-Oxy-androsterone, 11-oxy-etiocholanolone—combined values of 11-hydroxy and 11-carbonyl derivatives of androstane-3α-ol-17-one and etiocholane-3α-ol-17-one.

Muscular strength

The values obtained from the exercise tests are shown in Table 4. The mean premedication value was 59.9 seconds, with a range from 25 to 95 seconds. It was derived from two sets of tests, one month apart, before and after the administration of a placebo linguet daily. These two sets of values were in close agreement, the group means being less than two seconds apart.

After the onset of steroid medication the values rose rather rapidly during the first month (mean, 81.9 seconds) and more slowly thereafter, reaching a peak of 95.4 seconds after four months. The mean value in the next month was essentially the same—94.4 seconds. The net increases from the control level and the percentage increases show the same type of trend, the mean values at four months being 35.5 seconds and 65.17 per cent, respectively. Four subjects showed no consistent change.

After the medication was discontinued for four and a half months, the values, though lower than at the end of the medication period, were still

well above the initial levels, the average net increase being 25.6 seconds, and the average percentile increase, 47.1.

The increase in muscular strength must be attributed to the well-known anabolic effect of the steroids, particularly testosterone. It is presumed that this change, once induced, subsided very slowly, since the exercise test values were still elevated four to five months after the medication was discontinued.

Although the medication was effective in increasing consistently the en-

TABLE 4. INFLUENCE OF STEROIDAL THERAPY ON MUSCULAR STRENGTH (EXERCISE TIME) IN OLD MEN

Period		No. of subjects	Exercise time (sec.)		Percentage change from pre-treatment level
			Mean	Range	
Pre-treatment		14	59.9	33– 95	
During treatment	1 mo.	13	81.9	45–120	+47.3
	3 mo.	13	89.2	35–180	+58.8
	4 mo.	14	95.4	46–150	+65.1
	5 mo.	13	94.4	45–220	+62.0
Post-treatment	(4.5 mo.)	12	87.0	45–165	+47.1

durance of 10 of the 14 elderly subjects, there was a good deal of variation during the period of medication, both within and between individuals. By the end of the fourth month of medication there was a suggestive positive relationship between the initial level and the change in the weight-lifting time ($t = .16$, $P > .05$), *i.e.*, the longer the weight-lifting time, the greater the increment. Such a trend would imply that improvement was more possible when the muscular tissue was not irreversibly atrophic. This relationship was further indicated by the fact that the subjects who showed the greatest increases in endurance time at five months tended to display this trend as early as the first month after medication had begun. There was a suggestive trend in the relationship between the changes in endurance time from the initial level at the first month versus the changes at the fifth month ($t = .32$, $P > .05$). It would seem, then, that the increase in muscular strength following steroid medication tends to be a continuous and systematic process rather than an irregular one.

There is some relationship between the premedication exercise-time and age, as would be expected. The subjects in the eighth decade showed an

average value of 76.2 seconds, whereas for those in the ninth decade the initial mean was 54.1 seconds. The older subjects also tended to show the least increase in their exercise time after five months of medication, which again would be expected—the average increase for those in the eighth decade being 32.2 seconds, whereas for those in the ninth decade it was 23.7 seconds.

Blood pressure and weight

The effects of the medication on the blood pressure and weight are shown in Table 5. There was no effect upon the systolic and diastolic blood

TABLE 5. INFLUENCE OF STEROIDAL THERAPY ON BLOOD PRESSURE AND WEIGHT IN OLD MEN

Period		Systolic B.P. (mm. Hg)	Diastolic B.P. (mm. Hg)	Weight (lbs.)
Pre-treatment		142	71	144
During treatment	1 mo.	130	73	144
	3 mo.	139	74	143
	4 mo.	144	68	144
	5 mo.	131	68	148
Post-treatment	(4.5 mo.)	133	76	149

pressures for the group as a whole. There were initially 4 subjects in whom the blood pressure was over 150 mm. Hg, but all later showed some degree of reduction during the period of medication, with only 1 showing a sustained hypertension. One of these hypertensive subjects showed an initial diastolic pressure of 100 but this, too, fell to 90 later.

During the period of medication there was an average increase in weight of about 4 pounds (Table 2) during the last month of medication. One subject had a loss of weight (3 pounds), but all the others gained in varying amounts up to 11 pounds. There was no evidence of edema, but it is probable that some of this gain in weight was partly due to salt and water retention. There was no relationship between the initial weight and the change in weight after five months of medication. Discontinuance of the medication did not result in weight loss, as one would expect if it were largely due to fluid.

Psychologic, neurologic and encephalographic effects

The psychologic studies showed no significant changes as a result of the

medication. There seemed to be a decline in memory when tested on the Wechsler Memory Scale and the Stanford-Binet sentences, but this may have been due to further aging of the subjects or to technical factors in the reapplications of the test procedures. At any rate, no improvement was noted in concentration, effectiveness of learning, foresight or memory.

The hand-steadiness test scores were likewise unchanged. Apparently the neurologic factors controlling fine motion were unaffected by the steroid medication employed in this study.

Analyses of the encephalographic data showed no significant effects as a result of medication. There was a slight tendency toward a more frequent appearance of slow waves and a more diffuse distribution of the various wave frequencies, but these were all within the variation noted in previous periods without medication.

General effects

The subjects had been told initially of the general nature of the study and that it was designed to determine whether their well-being was favorably influenced. No further discussion was made until the end of the period of medication and the recheck four and a half months later. Because of their previous knowledge, their statements were taken with some reservations.

There was no strong enthusiasm regarding the effects at the end of the period of medication. Nine subjects felt somewhat stronger. Seven said that urinary force was improved. One stated that he had fewer "colds" than usual. Three subjects noted no difference in their general feeling as a result of medication. Of these, only 1 showed a moderate improvement in exercise time. Thus, in 2 of these 3 subjects, their feelings coincided with the results of the strength tests.

At the time of retesting after the medication was discontinued, 8 subjects stated that their feelings of well-being were maintained, 2 said that their strength had lessened, and 1 complained of diminished urinary force.

On the whole, insofar as subjective sensations could be trusted, the subjects' statements seemed to approximate the results of the strength tests.

DISCUSSION

The results of this study on steroid administration in aged men will be considered in their relation to two problems: (a) the physiologic effects and (b) the metabolism of the administered steroids.

Within the limits of the criteria chosen, no physiologic effects (exclusive of those on muscle) were observed during therapy. The concentration of blood and urinary inorganic ions, the urinary creatinine level, and the blood eosinophil count did not change significantly from pre-therapy levels.

The pre-therapy concentrations of these constituents in the aged subjects (with the exception of creatinine) corresponded to normal values irrespective of age. The low creatinine excretion (0.92 Gm. compared to the normal range of 1.6–1.8 Gm. per twenty-four hours) is explained by the relative muscular inactivity of the subjects. The data would indicate that the amounts of hydrocortisone and corticosterone administered were not sufficient to affect the sodium and potassium balance or the level of circulating eosinophils. Similarly, the reported ability of testosterone to cause some retention of sodium, potassium and inorganic phosphorus (10) was not observed. Since no determinations were carried out during the first few days following initiation of therapy, no data on any possible initial changes were obtained.

It is concluded that the quantities of steroids administered did not exert a marked action upon the physiologic indices investigated. However, these findings do not exclude the possibility of slight, but masked, effects. The suggested correlation between increased calcium and 11-oxygenated etiocholanolone excretion during treatment would point towards such possibilities. A complete metabolic balance study, in which such factors as diet and fecal excretion are controlled, will be necessary to detect and evaluate these slight steroidal activities.

The actual and expected recoveries of administered steroids during the first month of therapy were compared (Table 6). Insufficient data on the recovery of individual 17-ketosteroids for subsequent months of treatment precluded further comparisons. The recovery values for hydrocortisone and adrenosterone as 11-oxygenated-17-ketosteroids agreed well with the theoretical values. In contrast, the metabolic conversion of testosterone to androsterone and etiocholanolone differed from that in younger subjects (12). Androsterone excretion was 78 per cent higher and etiocholanolone 26 per cent lower than the calculated levels. These data suggest enzymatic changes in older men such that (a) the ratio of androsterone to etiocholanolone upon testosterone reduction is increased, or (b) there is increased conversion of etiocholanolone to etiocholane-3α,17β-diol.

It will be noted in Table 3 that the mean total 17-ketosteroid, androsterone and etiocholanolone concentrations in 4 subjects during the fifth month of therapy had decreased 14, 29 and 55 per cent, respectively, from the output during the fourth month. The mean total 17-ketosteroid excretion for all 11 subjects, however, did not decrease from the fourth to the fifth month of medication (Table 2). It is concluded that the observed decrease among the 4 subjects represents the results derived from an accidentally biased sample rather than a significant decrease.

From the foregoing data, it is evident that the administration of steroids does increase the muscular strength of older individuals. This effect

TABLE 6. COMPARISON OF URINARY 17-KETOSTEROID EXCRETION IN AGED MEN (70–91 YEARS) FOLLOWING STEROID ADMINISTRATION WITH THE EXCRETION IN YOUNG MEN (20–29 YEARS)

Steroid	Source	Urinary 17-KS in aged men (mg./24 hrs.)			Urinary 17-KS in young men† (mg./24 hrs.)
		Theoretical*	Pre-medication	Medication (month 1)	
Total 17-ketosteroids	Total	—	4.5	16.8	11.2
	From medication	—	0	12.3	—
Androsterone	Total	—	1.28	6.81	4.0
	From medication	3.00	0	5.53	—
Etiocholanolone	Total	—	1.20	5.63	3.8
	From medication	6.00	0	4.43	—
11-Oxygenated androsterone	Total	—	0.37	1.35	0.85
	From medication	1.02	0	0.98	—
11-Oxygenated etiocholanolone	Total	—	0.50	1.03	0.63
	From medication	0.63	0	0.53	—

* Calculated recovery of administered steroids.
† Dorfman (12).

is noted as early as one month and reaches a maximum by the fourth month of medication. It is more pronounced in the younger members of this group, probably because the atrophic processes associated with aging are less marked in these subjects. It is of great interest that this increased muscular strength persists, though to a less marked degree, for as long as at least four and a half months after discontinuance of the medication. There are no untoward effects, except for a slight gain in weight.

The failure of the medication to induce any psychologic or neurologic changes in aged subjects may be due either to a lack of effect on the central nervous system or to the presence of irreversible changes in that sphere. Such lack of effects, however, militates against the usefulness of these steroid substances in combatting the psychologic changes accompanying senescence, which are of wider import than the decreasing strength.

SUMMARY

A group of 14 men, aged 70 to 91, received daily for five months a steroid mixture composed of testosterone, adrenosterone, hydrocortisone acetate and corticosterone. At monthly intervals prior to, and during medication, urine and blood samples were collected for sodium, potassium, calcium, inorganic phosphate, blood eosinophil, urinary creatinine, and urinary 17-ketosteroid determinations. An additional specimen was analyzed five months after the end of medication.

No changes were observed in the serum and urinary concentrations of inorganic ions, in the blood eosinophil count, or in the excretion of creatinine. Urinary total 17-ketosteroid excretion increased with steroid administration from 4.5 to 17.1 mg. per twenty-four hours. This level was maintained throughout treatment and was followed by a subsequent decrease to pre-therapy values after cessation of medication. The excretion of 11-desoxy-17-ketosteroids differed from the expected output; 11-oxygenated steroid levels agreed well with the theoretical values. An exercise test demonstrated a significant improvement in muscular strength in 10 of the men. This improvement was maintained to a lesser degree for at least four and a half months after discontinuance of the medication. No change was noted in the psychologic status, neuromuscular coordination, or the encephalograms of the subjects. No untoward effects were observed.

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A SYSTEMATIC EVALUATION OF THE NELSON-SAMUELS PLASMA 17-HYDROXY-CORTICOSTEROID METHOD*

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INTRODUCTION

THE estimation of adrenocortical activity by the quantitative chemical measurement of blood hormone concentrations became practicable with the introduction of the Nelson-Samuels method in 1952 (1). The sensitivity and specificity required for the measurement of the minute quantities of free adrenocortical hormone in peripheral blood were achieved largely by the application of adsorption chromatography and by a micromodification of the Porter-Silber reaction for 17,21-dihydroxy-20-ketosteroids. The biologic importance of the method is emphasized by work from several laboratories identifying 17-hydroxycorticosterone, a 17,21-dihydroxy-20-ketosteroid compound, as a principal adrenocortical hormone in blood of humans and certain laboratory animals (2–6).

Some of the early attempts to reproduce this method were unsuccessful and rather extensive modifications were suggested by other workers (7, 8). In 1953 Nelson and Samuels published some additional technical details, acknowledged that difficulty had been encountered by others, and placed emphasis on the importance of the preparation of materials, particularly the chromatographic adsorbent (Florisil) (9). Although several laboratories have now reported success in using the method, some concern over its reproducibility continues to be expressed in the literature (10, 11). In view of this controversy, the present authors felt that a detailed evaluation of the precision of the method would be necessary before it could be used in a proposed long-term study of the regulation of corticotropin secretion. Also, there is a particular need for data on which to base the interpretation of results obtained with relatively low hormone concentrations, such as are found in dog and normal human plasma samples. Therefore, it is the purpose of this report to present the data obtained in a systematic analysis of the Nelson-Samuels method, with the viewpoint of defining

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the limits for interpretation of results and of determining the suitability of the method for the measurement of the low hormone levels observed in the dog or in the normal human subject.

METHOD

1. *Preparation of materials*

Ethyl alcohol. Absolute alcohol was allowed to stand with phenylenediamine dihydrochloride with occasional shaking for about a week. It was distilled in an all-glass distilling apparatus and only the fraction coming over at 78.8° C. was kept. This was used as a solvent for making the standard cortisone and hydrocortisone solutions.

Methyl alcohol. Merck reagent-grade methyl alcohol (100 per cent) was distilled over 2,4-dinitrophenylhydrazine before using.

Chloroform. Merck reagent-grade chloroform was redistilled over potassium carbonate no more than four hours before use and collected in brown bottles.

Florisil. The purchased product[1] was washed in small batches in running tap water for eight hours on two successive days. Over a period of the next three days it was washed with absolute ethanol five times and stirred frequently during each of the five washings. The alcohol was drained off as completely as possible and the Florisil placed in large evaporating dishes and dried in an oven at 120° C. for about sixty-six hours. After allowing the Florisil to come to room temperature it was stocked in brown bottles until needed.

The day before using, the Florisil was activated in a muffle furnace at 600° C. ± 25° for four hours and allowed to cool in the furnace overnight. Only enough Florisil for the day's use was activated. Any material remaining after the columns were packed was discarded. It was found that the Florisil does not retain its activation in a reliable manner, nor was any attempt made to use reactivated material. The temperature of activation is critical. Poor standard curves resulted from using Florisil which was activated at 650° C. or higher.

Glass wool. Pyrex borosilicate glass wool (#722 Corning) was washed in redistilled chloroform twice, dried in a hood at room temperature, and stored in a glass jar until used.

Coupling agent. Phenylhydrazine was recrystallized five times from ethyl alcohol and the final product was stored in a brown bottle in a desiccator.

A stock solution of dilute sulfuric acid (310 ml. of concentrated acid to 190 ml. of distilled water) was kept at room temperature. About ten minutes prior to use, 16 mg. of the recrystallized phenylhydrazine were added to 10 ml. of the dilute sulfuric acid. The mixture was shaken until the crystals were dissolved.

Standard solutions. The standard stock solutions were made from crystals of the free alcohol of cortisone and hydrocortisone in concentrations of 100 micrograms per ml. One ml. of the final concentration of the working standard contained 10 micrograms of the steroid.

2. *Procedure*

The details of the Nelson-Samuels method as it was followed during the course of these experiments are as follows:

A 10-ml. aliquot of heparinized plasma was placed in a 50-ml. glass-stoppered centrifuge tube and shaken for thirty seconds with 1.5 volumes of chloroform. The tube was

[1] Floridin Company, Warren, Pa.

centrifuged for ten minutes at approximately 3,500 rpm to break the emulsion and the clear chloroform layer was siphoned, by means of a serum lifter, into a 125-ml. Erlenmeyer flask. The extraction was repeated twice more and the total chloroform extract was evaporated to dryness in a 45° C. water bath under a stream of dry air. The resulting residue was picked up in 5 ml. of chloroform.

The 17-hydroxysteroid was separated from other steroid and chloroform-soluble materials present by passing the mixture through a chromatographic column. The column was shaped like a thistle tube. The upper bulb had a volume of 50 ml.; the tube was about 12 cm. long with an inside diameter of 11 mm., constricted at the lower end to about 4 mm. The column was prepared by placing a small wad of glass wool over the lower constriction and packing to a distance of 7 cm. up the tube with Florisil. When the Florisil was packed to the proper "tightness" the eluate flowed at a rate of approximately 2 ml. per minute. A second wad of glass wool was placed over the Florisil to prevent disturbing its surface during the addition of the sample and the eluents.

The Florisil column was washed first with 15 ml. of chloroform. The column was never allowed to get dry. As the meniscus reached the upper glass wool plug the 5-ml. portion of chloroform containing the sample was added to the column. The transfer was made using the most rigid analytical technique and the upper part of the column was washed several times to insure against loss of the sample by adherence to the walls of the column. As the meniscus of the washings approached the glass wool, 25 ml. of chloroform were added; this was followed by 25 ml. of 2 per cent methanol in chloroform. Both of these fractions were collected in a 125-ml. flask and discarded. Finally, a 45-ml. sample of 25 per cent methanol in chloroform was passed through the column and collected in a clean 50-ml. flask. This fraction, containing the 17-hydroxycorticosteroids, was evaporated to dryness on a 45° C. water bath under a stream of air. The sides of the flask were washed with chloroform to concentrate the steroid in the bottom of the flask and these washings were evaporated as before. The dry steroid residue was now transferred to a test tube with four 1-ml portions of methanol. The methanol was evaporated to dryness. The residue was dissolved in 0.2 ml. of methanol. After adequate shaking, 0.3 ml. of the phenylhydrazine-sulfuric acid reagent was added. The tube was shaken vigorously, stoppered, and incubated for one hour in a 60° C. water bath.

After incubation the material was transferred to microcuvettes and the optical density was read against a column blank in a modified DU Beckman spectrophotometer between 360 and 440 mμ. Fused silica microcells with a light path 10 mm. in length and 2.5 mm. in width were used. The spectrophotometer was equipped with a diaphragm attachment containing a 1.4-mm. hole which directs the light beam through the cuvette. The column blank was prepared by passing 5 ml. of chloroform through the chromatographic column and treating it in a manner identical to that used for the sample throughout the entire procedure. Results were calculated to terms of adsorption factor after the method of Allen (12). This calculation requires an optical density reading at the wave length where maximum light absorption occurs. Determination of this wave length was made by reading the optical density at every 2 mμ between 390 mμ and 410 mμ for cortisone and hydrocortisone after exposure to the Porter-Silber reaction. A family of curves for each of these compounds between 1 and 8 micrograms was constructed by plotting optical density against wave length. These curves show a plateau between 400 mμ and 406 mμ. The optical density at 410 mμ was smaller than the 400 mμ reading. Since the point of inflection occurred at 400 mμ this wave length was chosen as the peak absorption for all absorption factor calculations. This maximum differs from the 410 mμ

reported by Nelson and Samuels (1) but the deviation may be due to variation in the physical characteristics of the particular instrument used.

RESULTS

The complete Nelson-Samuels method resolves itself into three steps:

1. The colorimetric reaction.
2. The chromatographic purification.
3. The plasma extraction procedure.

The evaluation of the method will follow these convenient subdivisions.

I. *Evaluation of the colorimetric reaction*

On ten successive occasions, five sets of duplicates ranging in concentration from 1 to 8 micrograms, were pipetted from the working standard cortisone solution into test tubes, and evaporated to dryness in a 45° C. water bath under a stream of air. The residues were picked up in 0.2 ml. of redistilled methanol, coupled with the sulfuric acid-phenylhydrazine reagent, stoppered, and incubated for sixty minutes in a 60° C. water bath. The intensity of the resultant color complex was measured in a DU model Beckman spectrophotometer between 360 and 440 mμ. Data are shown in Table 1. It will be noted that absorption factors for members of each set of duplicates correspond remarkably well. The statistical evaluations for these data in terms of absorption-factor means, plus or minus the standard deviation, are listed in Table 2.

II. *Evaluation of the colorimetric plus the chromatographic steps*

Ten successive experiments were performed, in which duplicate aliquots of working standard cortisone solutions, ranging in concentration from 1 to 8 micrograms, were passed through Florisil columns and the Nelson-Samuels method followed as described under Method. Inspection of Table 3 will show that absorption factors for members of each set of duplicates are in extremely close agreement. The statistical analyses of these data in terms of absorption-factor means, plus or minus the standard deviation, for each concentration are shown in Table 4.

III. *Evaluation of complete method*

According to Allen (12), the validity of using the absorption-factor notation instead of optical-density readings is contingent on the fact that the total background absorption in terms of optical density is linear with respect to wave length.

The background interference has been studied here, using both dog plasma and human plasma. The combination of column blank, plasma

TABLE 1. DUPLICATE ABSORPTION FACTORS FOR CORTISONE BEFORE CHROMATOGRAPHY

Concentration (micrograms)				
1	2	4	6	8
0.043	0.096	0.159	0.228	0.288
0.040	0.093	0.144	0.222	0.270
0.037	0.070	0.152	0.227	0.299
0.035	0.081	0.154	0.228	0.321
0.055	0.082	0.160	0.230	0.300
0.043	0.092	0.169	0.228	0.303
0.042	0.112	0.190	0.257	0.346
0.048	0.102	0.186	0.256	0.343
0.039	0.083	0.158	0.236	0.319
0.039	0.082	0.158	0.236	0.304
0.042	0.093	0.179	0.267	0.376
0.039	0.092	0.179	0.263	0.362
0.038	0.071	0.170	0.242	0.317
0.036	0.075	0.170	0.245	0.312
0.040	0.081	0.157	0.240	0.324
0.036	0.077	0.154	0.231	0.302
0.038	0.078	0.154	0.233	0.315
0.041	0.074	0.159	0.226	0.300
0.036	0.065	0.142	0.216	—
0.033	0.076	0.149	0.209	—

TABLE 2. PRECISION OF COLORIMETRIC REACTION ONLY

Conc. cortisone (micrograms)	Absorption factor (mean ± s.d.)*	s.d. (micrograms)
1	0.040 ± 0.006	0.15
2	0.075 ± 0.010	0.25
4	0.162 ± 0.008	0.20
6	0.236 ± 0.008	0.15
8	0.317 ± 0.021**	0.50

* 0.05 probability, 19 d.f., $t = 2.093$.
** 0.05 probability, 17 d.f., $t = 2.11$.

TABLE 3. DUPLICATE ABSORPTION FACTORS FOR CORTISONE AFTER CHROMATOGRAPHY

Concentration (micrograms)				
1	2	4	6	8
0.042	0.069	0.138	0.202	0.277
0.032	0.068	0.135	0.201	0.268
0.033	0.063	0.149	0.208	0.259
0.031	0.063	0.141	0.195	0.253
0.037	0.068	0.135	0.197	0.261
0.026	0.062	0.132	0.205	0.269
0.035	0.061	0.127	0.194	0.251
0.033	0.044	0.117	0.197	0.251
0.032	0.068	0.130	0.205	0.253
0.030	0.063	0.121	0.206	0.259
0.032	0.058	0.130	0.186	0.244
0.034	0.056	0.121	0.181	0.257
0.032	0.059	0.132	0.186	0.242
0.032	0.062	0.126	0.195	0.268
0.028	0.064	0.133	0.205	0.278
0.036	0.072	0.134	0.202	0.274
0.031	0.063	0.164	0.211	0.269
0.026	0.063	0.156	0.206	0.266

TABLE 4. PRECISION OF COLORIMETRY AND CHROMATOGRAPHY

Conc. cortisone (micrograms)	Absorption factor mean ± s.d.*	s.d. (micrograms)
1	0.032 ± 0.008	0.23
2	0.062 ± 0.008	0.23
4	0.136 ± 0.010	0.30
6	0.200 ± 0.008	0.23
8	0.262 ± 0.015	0.45

* 0.05 probability, 19 d.f., t = 2.073.

blank and reagent blank comprises the background absorption. The latter two factors are negligible and the largest part of the background absorption is due to the column blank. However, when the optical density of the combined factors is plotted against wave length, the relationship between 360 mμ and 440 mμ closely approaches linearity.

The Nelson-Samuels method requires three extractions of the plasma with 1.5 volumes of chloroform. A series of 10-ml. aliquots of human plasma to which 4 micrograms of hydrocortisone had been added was extracted one, two, three and four times in order to determine whether further ster-

TABLE 5. PRECISION OF NELSON-SAMUELS METHOD

No. of extractions	Hydrocortisone added to 10 ml. plasma (micrograms)	17-OH steroid recovered (micrograms/10 ml. plasma)	
			Average
3	0	0.9	0.9
	0	0.8	
1	4.0	4.0	3.8
	4.0	3.6	
2	4.0	4.3	4.1
	4.0	3.9	
3	4.0	4.2	4.1
	4.0	4.0	
4	4.0	4.3	4.1
	4.0	3.8	

oid could be removed from the plasma with added chloroform washes. The data are presented in Table 5. These data show that three extractions with 1.5 volumes of chloroform are adequate for the removal of the steroid. Actually at 4 micrograms per 10 ml. of plasma, an increase of only 6 per cent is gained by more than one extraction.

Multiple aliquots of the same human plasma and dog plasma samples were analyzed on several days to test the duplicability of the method and the effect of storage on the plasma steroids. The plasma was stored in the refrigerator during the period of study. Data are shown in Table 6. The F test and the homogeneity of variance were applied to the multiple analyses of the human plasma. Critical value F, 5 per cent level, is 3.17 (3 and 10 d.f.). The calculated value for F, 1.11, is below the critical value; therefore the agreement between daily averages is acceptable as measured by the

TABLE 6. REPRODUCIBILITY OF NELSON-SAMUELS METHOD

Day	Concentration 17-OH corticosteroid (micrograms/100 ml. plasma)							
	Human	Mean	Dog 1*	Mean	Dog 2*	Mean	Dog 3	Mean
1	15 16 17	16	16 18 19	18	22 24 23	23	7 7 8 7	7
2	13 15 16 16 17	15	17 17 17	17	21 20 21	21	9 9 8 8 9	8
3	16 17 18	17						
4	15 17 18	16	15 17 17	16	20 21 22	21		
8			18 16 15 16 16 18	17	20 19 20	20		
14			17 16	17				
Total Mean		16		17		21		8

* Single i.v. injection of 40 mg. of ACTH one hour before blood withdrawn.

agreement between determinations within any one day. The critical 5 per cent level for Chi-square, d.f. equal to 3, is 7.80. The value calculated from the data, 0.012, is well within this upper limit, and it is concluded that the daily sets do not show differences in precision.

The critical 5 per cent level, d.f. equal to 1, for homogeneity of variance of the multiple dog plasma samples (No. 3) is 3.84. The calculated Chi-square value of 0.08 is well within this limit, indicating no difference in

precision. Data in Table 6 also show that the steroid level in the same sample is constant over a period of at least two weeks in both dog and human plasma. Plasma preserved in a deep-freeze is stable for at least nine weeks.

Recovery experiments were performed to see if three extractions with 1.5 volumes of chloroform were efficient in removing high concentrations of steroid from plasma. To 10-ml. aliquots of human plasma, 1, 2, 4, and 6 micrograms respectively of hydrocortisone were added. Duplicate samples were analyzed at each concentration. The recovery data are shown in

TABLE 7. RECOVERY OF 17-OH STEROID FROM PLASMA

Hydrocortisone added to 10 ml. plasma (micrograms)	Total steroid in 10 ml. plasma (micrograms)		Recovery of added steroid (micrograms)	Per cent recovery
		Average		
0	0.6	0.6	—	—
0	0.6			
1.0	1.5	1.5	0.9	90
1.0	1.4			
2.0	2.5	2.4	1.8	90
2.0	2.3			
4.0	4.1	4.1	3.5	88
4.0	4.0			
6.0	6.1	5.8	5.2	87
6.0	5.4			

Table 7. The data indicate that the extraction technique is capable of removing 90 per cent of the steroid present in plasma below levels of 6 micrograms per 10 ml. of plasma. At higher levels the recovery tends to fall off slightly.

DISCUSSION

The data show that the Nelson-Samuels method has a remarkable degree of precision and reliability. The standard deviation at the various concentration levels for the Porter-Silber reaction approaches a constant between 1 and 6 micrograms. The standard deviation for the 8-microgram level appears roughly twice as great as for the lower concentrations. However, the reason for the increase in variance is not inherent in the method.

It appears that the peak absorption at 400 mμ is so high that the optical density readings must be made in that part of the Beckman scale which represents the least sensitive output of the phototube.

Comparison of the mean values expressed in Table 2 with those in Table 4 will show that some steroid loss is attendant on the chromatographic step and its related operations (such as evaporation, methanol extraction and transfer). The mean absorption-factor values of the chromatographed samples for each concentration all fall between 80 and 85 per cent of the mean values of samples subjected to only the colorimetric procedure.

The data show that the combined chloroform extractions remove about 90 per cent of the 17-hydroxycorticosteroid present in the plasma. About 90 per cent of the total extracted steroid is taken out by the first 15 ml. of chloroform; the second extraction removes the remaining 10 per cent; and the third chloroform wash serves as a safety measure to insure that all traces of 17-hydroxysteroid which can be extracted are removed. Further washing of the plasma with chloroform fails to extract more 17-hydroxycorticosteroid. The partition coefficient seems not to have been exceeded—at least up to 6 micrograms per 10 ml. Very rarely do human or dog plasma aliquots contain more than this amount of 17-hydroxycorticosteroid.

It will be noted that absolute absorption factors vary from day to day. This variance is probably related to uncontrollable factors which may include atmospheric conditions, such as temperature and relative humidity. However, each concentration of 17-hydroxycorticosteroid is affected to the same extent by these variables. Hence the absorption factor-concentration relationship remains linear, although the slope of the line resulting from such a plot may change from day to day. Therefore, it is necessary to run a set of standards and construct a standard curve each time plasma steroid determinations are made.

SUMMARY

The Nelson-Samuels method is a difficult but remarkably reliable tool for the measurement of the minute amount of 17-hydroxycorticosteroids present in plasma. The preparation of materials is critical, particularly the washing and activation of the Florisil, and it is necessary to use rigid analytical technique in the handling of microgram quantities of hormone.

Based on analyses of known amounts of crystalline hormone, the precision of the method, at the 1-microgram level, is ±0.23 microgram. The standard deviation remains approximately constant up to 6 micrograms. Values for multiple aliquots of normal dog and human plasma samples invariably can be reproduced within this range.

It is felt that the limits described sanction the use of this method even

for the measurement of the low concentrations observed in many normal human subjects and in dogs.

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ENDOCRINE ACTIVITY IN ANOREXIA NERVOSA

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THE clinical aspects of anorexia nervosa are now well recognised but it is only in recent years that the endocrine dysfunction associated with the disease has been investigated.

From a series of 24 cases recorded elsewhere (1) 9 patients (8 females and 1 male) were selected for the present study in whom the disease had persisted without remission for a minimum of five years. Only 2 patients (females) who had recovered from anorexia nervosa could be persuaded to come into hospital to act as controls; these are referred to in the text as "the recoveries." The clinical details[1] of these 11 cases are presented in Table 1.

An attempt has been made to determine the activity of the anterior pituitary, adrenal cortex, thyroid and ovaries. The methods used are quoted in the text and the range of normal values accepted by the laboratory concerned are shown in the appropriate tables.

ANTERIOR PITUITARY ACTIVITY

Estimation of the urinary excretion of the follicle-stimulating hormone (FSH) (Method of Klinefelter *et al.* (2)) together with the insulin tolerance test (method of Fraser *et al.* (3)) were used for the assessment of anterior pituitary function. FSH estimations were carried out on two 24-hour specimens of urine collected fourteen days apart. In the 2 "recoveries," the specimens were collected during the eleventh and twelfth days of a 28-day menstrual cycle. The results are summarized in Table 2.

In 15 of the assays from the 9 patients with anorexia nervosa the FSH excretion was reduced, in 1 it was normal (Case 5) and in 2 it was increased (Cases 3 and 4). The results in Cases 3 and 4 are difficult to explain. Case 4 was probably not typical of anorexia nervosa; this was originally sus-

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[1] In addition to the tests for endocrine function, the following studies were carried out in each case, in order to exclude organic disease: x-ray examination of the chest, skull and gastro-intestinal tract (barium meal); blood count; blood Wassermann reaction; electrocardiogram; electroencephalogram; bromsulphalein excretion test; urinary dilution and concentration test; urinalysis; urea clearance; plasma protein level; blood urea level; glucose tolerance test; and fractional test meal.

TABLE 1. CLINICAL DATA OF THE 11 PATIENTS INVESTIGATED

Case No.*	Sex; and age at onset	Infant feeding difficulties	Age at onset of menstruation	Psychologic shock preceding symptoms	Loss of weight at onset of amenorrhea (lbs.)	Anorexia admitted by patient	Emaciated (E), Thin (T), Normal (N)	Pubic & axillary hair normal	Abnormal hair on trunk or limbs	Breasts preserved	Peripheral cyanosis	Edema of legs	Nocturia	Blood pressure (mm.Hg)	Pulse rate per minute	Age‡	Height (ins.)‡	Weight (lbs.)‡	Weight as % of standard‡	Duration of disease (yrs.)‡
1	F.21	−	12	+	<14	−	E	+	+	+	+	−	+	105/70	60	30	64	74	59	9
2	F.21	+	14½	−	<14	+	E	+	−	+	−	−	−	110/55	55	45	71½	74½	47	24
3	M.20	+		−	−	+	E	+	−	+	−	−	+	130/90	65	33	71½	95	58	13
4	F.17	−	16	−	<14	−	E	+	−	+	+	+	+	125/80	80	38	66	84	62	21
5	F.15	−	14½	D†	<14	−	T	+	−	−	+	−	−	95/70	60	33	64	93	73	18
6	F.18	−	14	D	28–42	−	T	+	−	+	−	−	−	110/80	65	35	65½	92½	70	17
7	F.26	−	14	−	56	−	T	+	−	+	−	+	−	80/60	60	43	68	86	59	17
8	F.16	−	14	D	56	−	E	+	+	+	−	+	−	85/45	55	21	66	72	55	5
9	F.22	−	12	+	<14	+	N	+	−	+	−	−	−	130/85	80	47	64½	105	75	25
11	F.23	−	12½	−	14–28	+	T	+	−	+	−	+	−	85/45	80	30	67	118	87	1½
12	F.24	+	13	+	<14	+	T	+	−	+	−	−	−	115/80	44	39	67	122	87	2

* Patients 1–9 still have anorexia nervosa. Patients 11–12 have recovered from anorexia nervosa. Standard weights were obtained from Kemsley (50). All heights include 1½ inches for shoes and all weights include 6 pounds for light clothing.

† D = Voluntary dietary restriction.

‡ At the time of the present investigation.

TABLE 2. INVESTIGATIONS FOR ANTERIOR PITUITARY ACTIVITY

Urinary FSH excretion and results of insulin tolerance test in 9 patients with anorexia nervosa and in 2 "recoveries" (Cases 11 and 12)

Case No.	FSH excretion (m.u. per 24 hrs.)	Insulin tolerance
1	<4	H.U.+I.S.*
	<4	
2	<4	Normal
	<4	
3	<4	H.U.
	64–128	
4	<4	I.S.
	32–64	
5	<4	I.S.
	4–8	
6	<4	Normal
	<4	
7	<4	Normal
	<4	
8	<4	H.U.+I.S.
	<4	
9	<4	H.U.
	<4	
11	4–8	Normal
	4–8	
12	4–8	Normal
	4–8	
Normal Range	4–8	*See* Fraser *et al.* (3)

* H.U. = Hypoglycemia unresponsiveness. I.S. = Insulin sensitivity.

pected from the physical signs. In Case 3 (the only male of the series) FSH excretion was extraordinarily high—a most unusual finding in anorexia nervosa. If confirmed, an examination of seminal fluid and a testicular biopsy would be required to assess testicular function before any conclusion could be drawn (4).

MacGregor (5) found that gonadotropins were absent from the urine in 7 out of the 8 cases of anorexia nervosa he investigated. Similar findings were reported by Pardee (6) and Fraser *et al.* (7), who found no FSH in 3 out of 4 of their cases. Berkman (8) considered that the gonadotropins were greatly reduced or absent in all cases. This has been confirmed more recently by Sunderman and Rose (9), White and Moehlig (10) and Hubble (11). An interesting case was reported by Reiss (12), who found no gonadotropins in a patient when the weight was 54 pounds; but after recovery,

when the weight was about 126 pounds, gonadotropins were present in the urine.

McCullagh and Tupper (13) on the other hand, using a modification of Friedman's test, found that out of 18 patients investigated, there was an

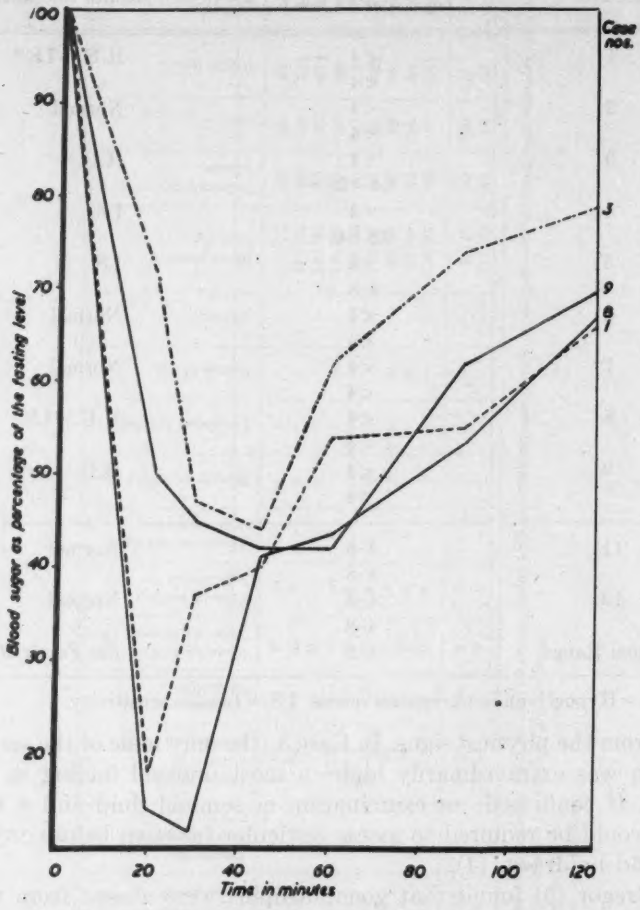

FIG. 1. Hypoglycemia unresponsiveness in 4 cases of anorexia nervosa.

excess of urinary gonadotropins in 7 and a measurable quantity in 3. From these findings they concluded that there was no reduction of anterior pituitary function in anorexia nervosa. Bartels' findings (14) supported those of McCullagh and Tupper, for he demonstrated a normal gonadotropin excretion in 13 of the 14 patients investigated. These results, however, are

of less significance when it is realized that 5 of these patients were menstruating regularly and a sixth had at least one normal period while under investigation.

Amenorrhea is frequently an early symptom in anorexia nervosa, sug-

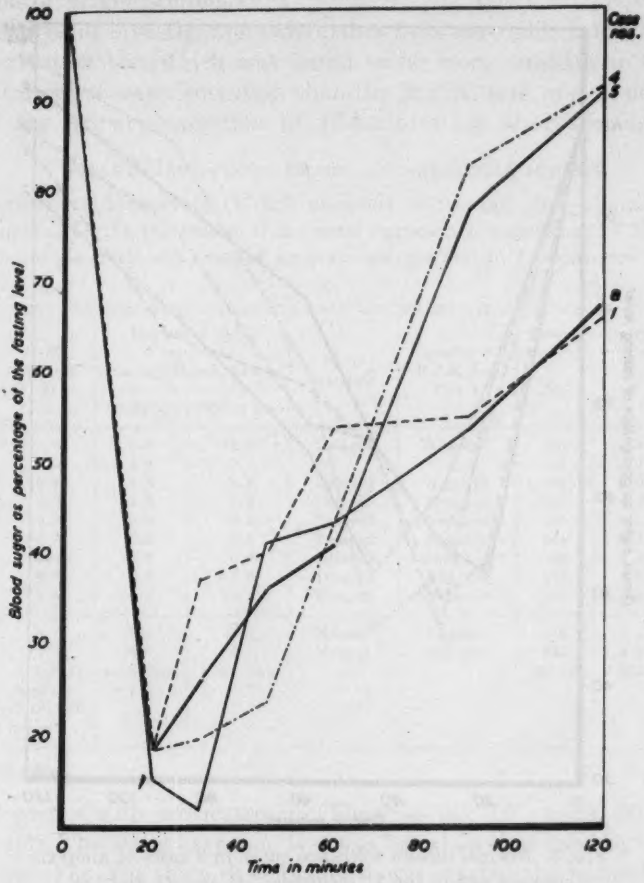

FIG. 2. Insulin sensitivity in 4 cases of anorexia nervosa, 2 of which also show hypoglycemia unresponsiveness.

gesting that it is a manifestation of anterior pituitary deficiency rather than the result of inanition due to undernutrition. This is in keeping with the reduced excretion of FSH and other gonadotropins, which is the common finding in anorexia nervosa.

Hypoglycemia unresponsiveness and increased insulin sensitivity may

occur in anorexia nervosa. In the present series hypoglycemia unresponsiveness was found in 4 of the 9 cases (Fig. 1). Similar results were obtained by Fraser *et al.* (3) in 3 of their 5 cases. A steep fall of the blood sugar level, probably due to insulin sensitivity, was referred to by Wilson *et al.* (15) but no details were given. In the present investigation this was

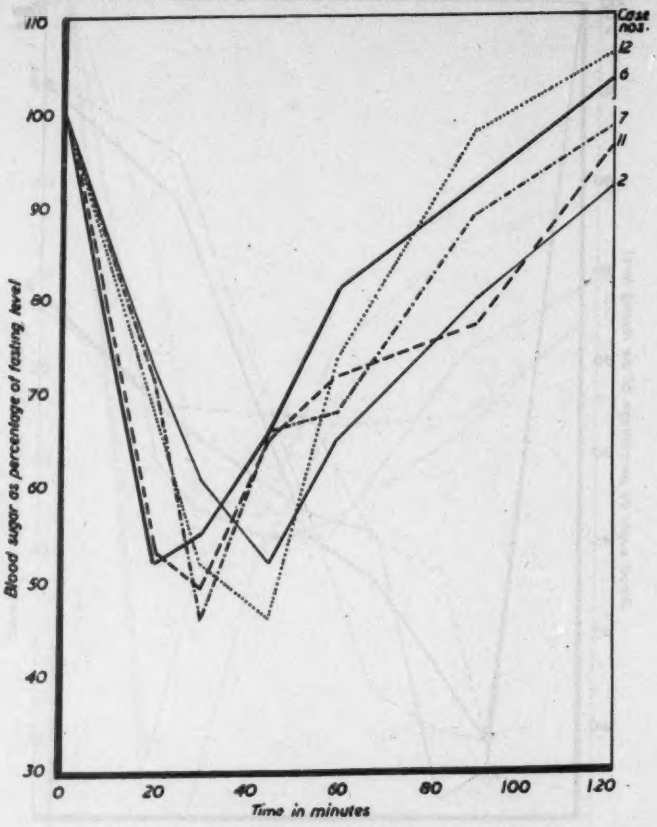

FIG. 3. Normal insulin tolerance curve in 3 cases of anorexia nervosa and in the 2 "recoveries" (Cases 11 and 12).

seen in 4 cases; 2 of these also had hypoglycemia unresponsiveness (Fig. 2). In 3 cases the insulin tolerance curve was normal (Fig. 3).

The reduced excretion of FSH and the abnormal findings with the insulin tolerance test in anorexia nervosa suggest some suppression of anterior pituitary function. These results, however, must be interpreted with caution, especially as methods for assaying the circulating adrenocorticotropic hormone (ACTH) and the thyroid-stimulating hormone (TSH) were not available.

ADRENOCORTICAL ACTIVITY

The only investigations which had previously been used to assess adrenocortical activity in anorexia nervosa were the Robinson-Power-Kepler test (R.P.K.), estimations of the serum electrolyte levels, and the urinary excretion of 17-ketosteroids.

In the present investigation two further tests were added: 1) a simplified water excretion test which was found to be more sensitive in detecting minor degrees of water retention than the R.P.K. test, and 2) determination of the urinary excretion of 17-ketosteroids after stimulating the

TABLE 3. INVESTIGATIONS FOR ADRENOCORTICAL ACTIVITY

The urinary 17-ketosteroid (17-KS) excretion before and after administration of corticotropin (ACTH), the results of the water excretion test and the R.P.K. test, and serum electrolyte levels in 9 cases of anorexia nervosa and in 2 "recoveries" (Cases 11 and 12).

Case No.	17-KS excretion (mg./24 hr.)	Increase of 17-KS excretion after ACTH (mg./24 hrs.)		Water excretion	Results of R.P.K. test Part 1	Serum electrolytes (mEq./L)		
		1st 24 hrs.	2nd 24 hrs.			Na	K	Cl
1	5.9	6.8	13.4	Delayed	Negative	140	4.4	100
2	4.9	4.9	9.0	*	*	138	4.9	96
3	10.9	4.5	8.8	Delayed	Negative	140	5.0	100
4	9.2	4.9	9.4	Delayed	Negative	144	4.2	105
5	4.5	5.0	9.0	Delayed	Negative	140	4.1	106
6	5.7	2.3	4.9	Delayed	Negative	144	5.2	98
7	2.8	4.0	6.4	Delayed	Negative	146	4.9	104
8	8.0	8.0	18.0	Delayed	Negative	142	3.8	100
9	4.4	5.0	16.2	Delayed	Negative	142	4.8	106
11	6.7	6.3	23.2	Normal	Negative	138	4.5	106
12	11.5	4.9	8.9	Normal	Negative	139	4.7	97
Normal range	6.0–18.0 (females) 10.0–25.0 (males)	more than 4.4	more than 8.7			136–147	3.9–5.2	97–107

* Test not made.

adrenal cortex with corticotropin (Thorn *et al.* (16), using 80 units of ACTH-gel). The water excretion test was based on that used by Chalmers and Lewis (17). For three days before it was started the patient's intake of fluid was a minimum of 4 pints a day. The test was abandoned if there was any diarrhea or vomiting during this period. After a water load of 1 liter, which was drunk in fifteen minutes, the volume of urine excreted in the following ninety minutes was measured. The urinary 17-ketosteroids were estimated by the method of Talbot *et al.* (18), and the R.P.K. test was carried out according to Robinson *et al.* (19). Serum sodium and potassium levels were determined by flame photometry (20) and chlorides by the method of Van Slyke and Hillier (21). Results are summarized in Table 3.

The results of the R.P.K. test, and the determinations of serum electrolytes and urinary 17-ketosteroids in the present series agree closely with those obtained by other authors.

In anorexia nervosa, the R.P.K. test yields normal results. The serum sodium, potassium and chloride levels are generally normal. Sunderman and Rose (9) found the concentration of serum chloride to be low in their case, but recorded a normal response of the serum levels after intravenous

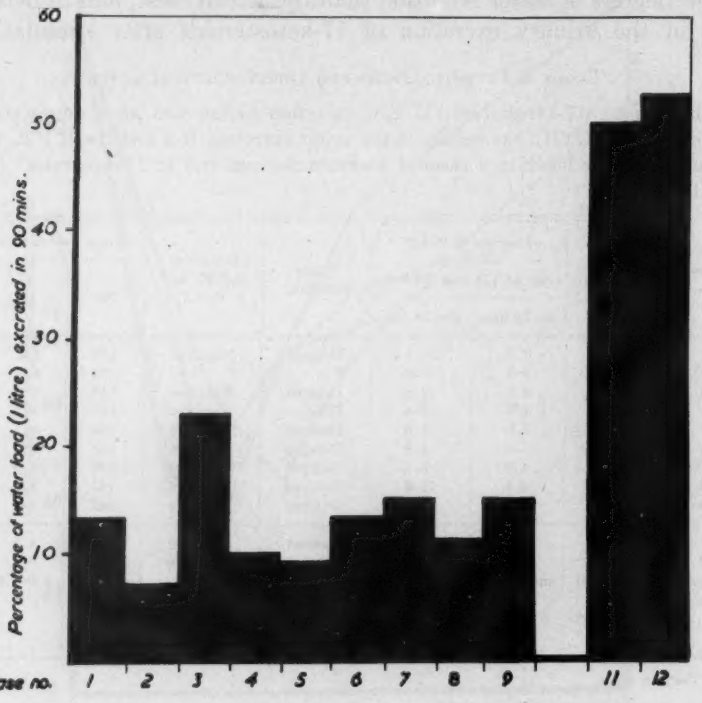

FIG 4. Water excretion in 9 cases of anorexia nervosa and in the 2 "recoveries" (Cases 11 and 12) after an initial water load of 1 liter.

administration of sodium chloride. Davies (22) and Stephens (23) reported a normal electrolyte response to sodium chloride restriction in patients with anorexia nervosa.

The urinary 17-ketosteroid excretion was low in 6 cases (67 per cent) of the present series. This agrees closely with the findings in 21 cases collected from the literature (2, 7, 9, 10, 24–26). In 13 (62 per cent) of these 21 cases the 17-ketosteroid excretion was below normal.

Water excretion, tested by the method described, was delayed in all cases examined (Fig. 4). Two previous authors have made similar observations (8, 27). It would appear, therefore, that there is delayed water ex-

cretion in anorexia nervosa but that the R.P.K. test is not sufficiently sensitive to demonstrate it. This delayed excretion, however, is not a specific test for adrenocortical function. Patients with anorexia nervosa may have abnormalities of water absorption, or may excrete a decreased quantity of solutes. In either event, there would be a delay in the excretion of water which would not directly implicate abnormal adrenocortical function.

After administration of corticotropin the urinary excretion of 17-ketosteroids became normal in the majority, if not in all cases investigated. There is no doubt, therefore, that even in severe cases in which symptoms have persisted for many years, the adrenal cortex is still able to respond normally to large doses of corticotropin. Treatment of the condition with this hormone, however, meets with indifferent success (28). The absence of irreversible change is also shown by the patient's ability to excrete normal quantities of 17-ketosteroids after recovery (12). In addition to the 2 "recoveries" a further patient, who is not included in the present series, also demonstrated this point. The 17-ketosteroid excretion was 1.4 mg. per twenty-four hours when the patient weighed 70 pounds. After recovery, the excretion increased to 8.8 mg. and the weight to 149 pounds. Similar figures were obtained when recovery followed leucotomy (29, 30). This was also personally observed in a case not included in the present series. Before leucotomy, when the patient weighed 66 pounds, the excretion of 17-ketosteroids was 3.5 mg. per twenty-four hours; after operation, when weighing 158 pounds, the excretion of 17-ketosteroids had risen to 16.0 mg. per twenty-four hours. A temporary reduction in 17-ketosteroid excretion is also seen in undernutrition. Landau *et al.* (31) demonstrated a 50 per cent reduction in 4 adults (3 men and 1 woman) after four days of starvation. The factors called into play in both undernutrition and anorexia nervosa are unknown. There are many similarities between the two conditions and as yet there is no contradiction of Sheldon's (32) hypothesis that suppression of the anterior pituitary occurs in both, and that this, as suggested by Thorn *et al.* (33), accounts for the decreased adrenocortical activity. The decrease, however, cannot be great; otherwise values for the R.P.K. test and serum electrolyte levels would be abnormal and the adrenal cortex would be unlikely to respond normally to stimulation with corticotropin. It is concluded, therefore, that in anorexia nervosa some hypofunction of the adrenal cortex occurs and that it probably results from decreased anterior pituitary activity, with reduced secretion of corticotropin.

THYROIDAL ACTIVITY

Assessment of thyroidal activity in anorexia nervosa has so far been confined to measurements of the basal metabolic rate (BMR). In the present investigation, radioactive iodine studies (method of Tait *et al.*

(34)) and serum cholesterol estimations (method of Schoenheimer and Sperry (35)) have also been used. Facilities for measuring the circulating protein-bound iodine were not available. The results are summarized in Table 4.

The BMR, which is an indirect measurement of thyroidal activity, is difficult to interpret in a complex metabolic disorder like anorexia nervosa. The results obtained in the present series, using Benedict-Roth apparatus

TABLE 4. INVESTIGATIONS FOR THYROIDAL ACTIVITY

Radioactive iodine studies, BMR, and serum cholesterol level in 9 cases of anorexia nervosa and in 2 "recoveries" (Cases 11 and 12).

Case No.	I^{131} clearance	Thyroidal I^{131} uptake in 24 hrs. (% of initial dose)	BMR (% of standard)	Serum cholesterol (mg./100 ml.)
1	30	32	−27	190
2	25	28	−26	260
3	14	9	−28	150
4	<10	4	0*	120
5	22	39	−25	270
6	25	46	−25	220
7	15	36	−14	280
8	<10	35	−33	169
9	<10	34	−13	210
11	52	48	− 8	190
12	30	40	− 8	210
Normal range	25–60†	40–60†	+15 to −15	150–280

* This patient would not co-operate.

† Derived from a study of over 450 patients during the previous five years at the Middlesex Hospital.

with recording kymograph, agree closely with those of other writers (5, 13, 26, 36, 37). The mean BMR of the 8 cases investigated was −24 per cent with a range of −13 to −33 per cent. The method and standards used were those of Robertson and Reid (38).

The cause of the depressed BMR in anorexia nervosa has never been adequately explained. Berkman (8) considered that it was dependent upon the duration of the disease, the degree of inanition and the level of the BMR before the onset of symptoms. Both he and Bartels (14) failed to find a simple relationship between the BMR and loss of body weight. Göbel *et al.* (39), on the other hand, found that the depression of the BMR in starvation was directly proportional to the loss of body weight

until from 20 to 24 per cent of the total weight had been lost. If loss of weight continued below this figure there was no longer a simple relationship. This may explain the findings in anorexia nervosa and account for the absence of a simple relationship in some long-standing cases.

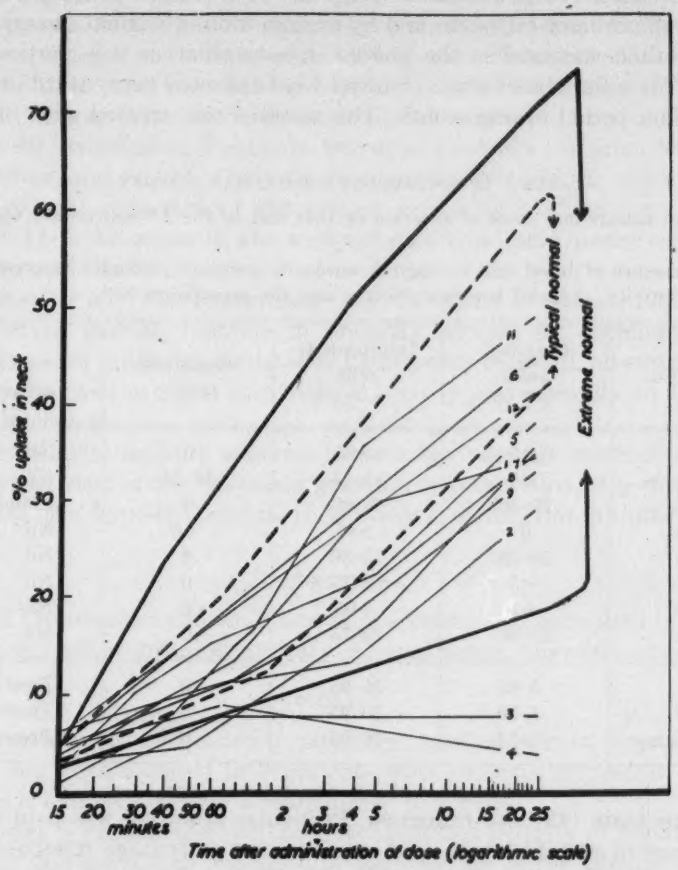

FIG. 5. The thyroidal I^{131} uptake in 9 cases of anorexia nervosa and in the 2 "recoveries" (Cases 11 and 12).

The serum cholesterol level was normal in all cases. This was expected, as in mild hypothyroidism an increase in serum cholesterol concentration is unusual (40).

From the present study it appears that a depression of the BMR and I^{131} clearance occurs in nearly all cases. Reduction of the thyroidal I^{131} uptake is a less constant finding (Fig. 5). The results of the radioactive iodine studies alone, indicate some supression of thyroidal activity (41),

but from the present work it is impossible to say whether this is the sole factor which accounts for lowering the BMR.

OVARIAN ACTIVITY

In anorexia nervosa, ovarian activity has been studied both by biologic assay of the urinary estrogens and by examination of vaginal smears. The latter method was used in the present investigation, as it is particularly suitable for serial observations. Smears were collected every third day for a minimum period of one month. The material was treated with Shorr's

TABLE 5. INVESTIGATIONS FOR OVARIAN ACTIVITY

Vaginal smears in 8 cases of anorexia nervosa and in the 2 "recoveries" (Cases 11 and 12).

The presence of basal cells in vaginal smears is a sign of profound suppression of estrogen activity, if found between puberty and the menopause (44).

Case No.	Cornified cells (%)	Intermediate cells (%)	Basal cells (%)	Cyclic activity
1	<5	>95	0	Nil
2	<5	45–55	45–55	Nil
4	20–40	60–80	0	Present
5	0	>95	<5	Nil
6	20–25	75–80	0	Nil
7	<5	>95	0	Nil
8	<5	>95	0	Nil
9	25–30	70–75	0	Nil
11	5–65	35–95	0	Present
12	5–70	30–95	0	Present
Normal range	5–70	30–95	0	Present

trichrome stain (42) and examined. Particular attention was paid to the percentage of cornified cells, and whether this percentage remained constant or showed cyclic variation (43–45). The results are summarized in Table 5.

As amenorrhea or severe oligomenorrhea was present in all the 8 female patients investigated, it was expected that ovarian activity would be absent or greatly reduced; this was so in 5 cases. The remaining 3 (Cases 4, 6 and 9) showed subnormal activity. Two of these 3 patients were apparently nearing recovery, as, when they were investigated, the body weight was considerably above the lowest weight recorded during their illness. One of these (Case 9) menstruated normally three times after leaving the

hospital, thus ending a period of amenorrhea which had lasted for twenty-two years. Case 4 was the least typical in the series, both from the clinical and the laboratory point of view; and, as the presence of urinary FSH was demonstrated, it was not surprising that there was some degree of ovarian function.

The present findings are in general agreement with those of other authors. Using biologic methods for assaying the ovarian hormones, MacGregor (5) found them to be absent from the urine in 8 out of 9 cases, and McCullagh and Tupper (13) noted reduced urinary estrogens in 11 out of 13 cases. Pardee (6) investigated 2 patients, one of whom had no ovarian hormone in the urine, and the other only small quantities. Sunderman and Rose (9) reported great reduction of the urinary estrogens in their case, as did Bartels (14) in his patients who were not menstruating. Urinary estrogens were absent in both cases reported by Hubble (11).

Rahman *et al.* (46), Richardson (47), Berkman (8, 48) and Moulton (49) assayed ovarian function in anorexia nervosa by examination of vaginal smears. Although no detailed figures were reported, all agreed that there was a great decrease in estrogenic activity and generally an absence of cyclic changes.

Patients suffering from anorexia nervosa have greatly reduced ovarian activity. In view of the decreased gonadotropin excretion, it seems probable that the hypo-ovarianism is secondary to anterior pituitary deficiency.

CONTROLS

The 2 "recoveries" showed a normal response to all tests used to assess the activity of the anterior pituitary, adrenal cortex, thyroid and ovaries.

SUMMARY

The activity of the anterior pituitary, adrenal cortex, thyroid and ovaries has been assessed in 9 patients with anorexia nervosa in whom symptoms had persisted for a minimum of five years.

Evidence is presented which suggests decreased activity of the anterior pituitary and associated reduction in the function of the adrenal cortex, thyroid and ovaries.

Normal activity of these glands was found in 2 other patients who had recovered. This suggests that no irreversible change had occurred during the course of the disease.

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HIRSUTISM IN INFANTILE THYROID INSUFFICIENCY

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NOT infrequently, we have seen an excessive growth of the body hair in infantile thyroid insufficiency. This anomaly of hair growth is most marked on the back, and least on the limbs. It occurs in both sexes, and in patients ranging in age from the first months of life up to adulthood. The most interesting fact is the disappearance of this hirsutism along with the clinical picture of thyroid insufficiency, following the administration of desiccated thyroid.

For the sake of brevity, we have chosen 3 histories of this type of hirsutism from a total of more than 10 filed at our Institute.

CASE HISTORIES

Case 1

J.S.Q. (No. 104), a female child aged 10 years 11 months, was born at term, with abundant hair and a great deal of lanugo all over the body, even on the face. According to data supplied by the mother, symptoms of thyroid insufficiency were present from birth (*e.g.*, apathy, constipation, delay in physical and mental development, first teeth at two years, head not held up until two years, walked at six years). We examined the patient for the first time when she was 10 years 11 months of age, on March 3, 1947, and found a typical case of infantile myxedema, with puffy expressionless facies (Fig. 1a), hoarse voice, pot belly and umbilical hernia. Her height was 101 cm., and skeletal age 4 years. There was marked growth of hair on the back as far down as the sacrum, and a much lighter growth of hair on the limbs (Fig. 1b). The skin was cold and dry. The blood cholesterol level was 240 mg. per 100 ml.

On March 14, 1947 treatment was started with 5 centigrams (about 1 gr.) of desiccated thyroid every other day. With this medication there was definite and progressive improvement of the myxedema (Fig. 1c) and also of the hirsutism (Fig. 1d).

Case 2

J.J.A.F. (No. 8), a boy aged 8 years 6 months, was born at term. From birth he presented an abundant growth of hair, predominantly on the back. Since his first months of life he was seen to suffer from the cold, to be apathetic, constipated and stunted in physical and mental development. At the age of 3 years goiter appeared. Examined when eight and a half years of age, on November 20, 1936, he presented a typical picture

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FIG. 1. Case 1. **a** and **b**—before treatment; **c** and **d**—after twelve months of treatment with desiccated thyroid.

of myxedema—cold dry skin; infiltrated facies; scanty, stiff, dry hair; a small umbilical hernia; persistence of milk teeth; and about a 2-year delay in ossification. The goiter (Fig. 2a) was diffuse, soft, and of medium size. There was a remarkably thick growth of hair on the back, extending from the midline outwards, and also on the external aspects of the forearms (Fig. 2b).

On November 24, 1936 treatment was started with 5 centigrams (about 1 gr.) per day of desiccated thyroid; after ten days the dosage was increased to 10 centigrams per day.

FIG. 2. Case 2. **a** and **b**—before treatment; **c** and **d**—after eighty-six days of treatment with desiccated thyroid.

On January 27, 1937 the improvement in myxedema was evident, and by February 18, *i.e.*, after eighty-six days of treatment, the hair had disappeared from the back (Fig. 2d) and there was no goiter (Fig. 2c).

Case 3

R.A.M. (No. 5188), a girl aged 13 years 8 months, was born at term. The gain in stature had been slow, dentition was delayed, and she had walked at two years. There was mental deficiency. She was placid but irritable, hardly ever cried, suffered from the cold, and was habitually constipated. Examined for the first time when she was 13 years and 8 months old, in November 1940, she presented the general appearance of congenital myxedema. Her height was 115 cm. (32 cm. below the normal minimum). She showed typical puffy facies, dental delay, pot-belly, small umbilical hernia, and dry, pale yellowish skin. There was a moderate amount of hair on the back, including the lumbar and sacral regions. On the limbs hirsutism was less marked. The blood cholesterol level was 287 mg. per 100 ml. Skeletal age was about 3 years, and there was great delay in the development of ossification centers; those present were coarse and irregular.

On December 14, 1940 treatment was begun with 5 centigrams (about 1 gr.) of desic-

cated thyroid. There was progressive improvement in the picture of thyroid insufficiency. By June 28, 1941 the hirsutism had disappeared and still further improvement in the myxedematous symptoms had taken place.

DISCUSSION

These 3 cases show clearly that the hirsutism in these patients was due to thyroid insufficiency. In a paper published in 1937, Mussio Fournier and Cerviño reported on the favorable effect of desiccated thyroid in this type of hirsutism (1). Perloff (2) has recently published a report on 4 cases, also showing plainly the effect of desiccated thyroid on the hirsutism of patients with infantile thyroid insufficiency.

We do not propose to explain the physiopathology of this abnormal hirsutism. However, merely as a hypothesis, it might be regarded as a persistence of lanugo. This supposition is in harmony with certain facts: in the first place, in some of our patients, we were able to ascertain that the hairiness in question was present at birth; in the second place, this hirsutism was most pronounced on the back and limbs, the usual sites for lanugo.

SUMMARY

Three cases are reported of congenital myxedema, presenting hirsutism as the most interesting clinical feature. This anomaly tended to disappear, together with the other clinical signs and symptoms of myxedema, after treatment with desiccated thyroid.

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Letters to the Editor

INSULIN-SPARING SULFONAMIDES*

To the Editor:

The following is a brief report of certain preliminary metabolic and clinical observations relating to the insulin-sparing sulfonamides.

In the initial reports relating to experimental and clinical effects of 1-butyl-3-*p*-aminobenzene sulfonyl urea (BZ-55), under the conditions in which the material was administered, a significant sustained hypoglycemic effect was demonstrated only in normal subjects and in patients with relatively mild diabetes (1).

Our data thus far accumulated deal for the most part with severe diabetes, inasmuch as it seemed to us desirable to determine at an early date whether patients in this group would prove to be relatively or absolutely unresponsive to these "insulin-sparing" sulfonamides. The observations were made in 5 such patients, in 1 pre-clinical female diabetic, and in 1 obese patient with mild diabetes. Two sulfonamides[1] were used—BZ-55, and a compound identical except for substitution of the amino group by a methyl group on the benzene ring (Orinase).

PRECISELY CONTROLLED STUDIES

In Figure 1 are shown studies in 2 male juvenile diabetics (VGLE and JJON). These patients were maintained on a quantitatively constant regimen during the first eighteen days of the study, and thereafter on a diet which was constant in a dietetic sense but not in a quantitative chemical sense. Of interest are the following:

1. Both patients appeared to have a transient inhibition of functional activity of the thyroid in terms of 24-hour radioactive iodine (I^{131}) uptake. In neither patient was the uptake determined before the beginning of the study. Neither patient showed any clinical evidence of thyroid dysfunction at any time. The early return toward normal of the thyroidal I^{131} uptake may be related to decreased dosage of sulfonamide.

* Supported in part by grants from The Upjohn Company and from Eli Lilly & Company.

[1] Grateful acknowledgment is made to Dr. C. J. O'Donovan, The Upjohn Company, for supplies of U-2043 (Orinase) and U-6987 (BZ-55); and to Dr. W. R. Kirtley, The Lilly Research Laboratories, for supplies of "BZ-55."

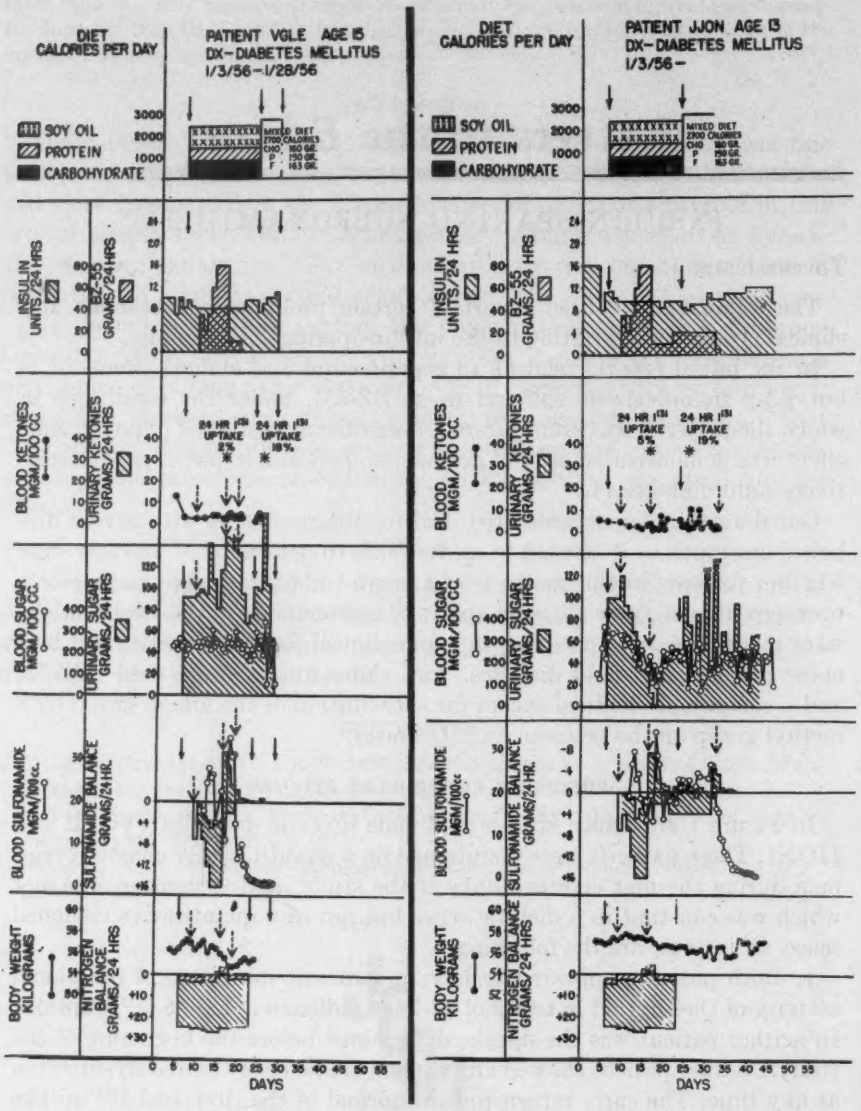

FIG. 1. Observations in 2 patients maintained on essentially identical metabolic programs. The distinctly different urinary sugar responses to sulfonamide are apparent.

In the balances, intake is charted down from the zero line. Output is charted upward from the lowermost portion of the intake. In the case of nitrogen balance, the lower hatched area is urinary nitrogen, and the upper hatched area is fecal nitrogen. Negative nitrogen balance is present only when the hatched area is above the zero line.

2. In neither patient was there any significant increase in the level of blood ketones, despite major glycosuria in Patient VGLE.

3. In Patient JJON (insulin and diet remaining constant), during the period of intensive sulfonamide administration, there was a profound decrease in blood sugar level and in glycosuria. Patient VGLE, treated identically, showed no decrease; instead, there was an increase in glycosuria, with little change in hyperglycemia.

4. Major fluctuations in blood free-sulfonamide levels were noted during periods of constant medication; and at times there was a temporary increase in blood levels, associated with a decrease in dosage.

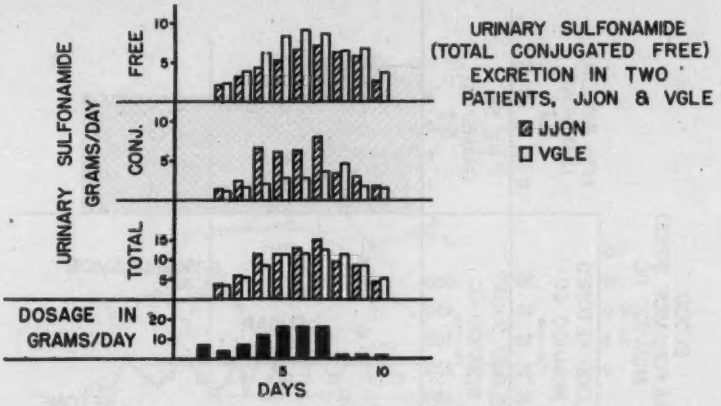

FIG. 2. Striking differences in free and conjugated urinary sulfonamides in Patients JJON and VGLE, despite approximate equality of total sulfonamide excretion. (Nearly 100 per cent of administered sulfonamide was recovered in the urine.)

5. Nitrogen balance patterns were surprisingly similar in the 2 boys, despite the extreme difference in glycosuria.

6. The *total* urinary sulfonamide excretion patterns were similar in the 2 subjects, but the *free* and *conjugated* urinary sulfonamide levels were distinctly different (Fig. 2).

7. Interesting changes in sodium and potassium balance were noted. These will be reported in a later paper.

Sulfonamide was discontinued in Patient VGLE because of a progressive fall in the total number of white blood cells, attributable largely to decreased circulating granulocytes. During the administration of sulfonamide, Patient JJON manifested a papular rash involving particularly the face. This disappeared promptly with decreased dosage. Except for moderate malaise during the period of most intensive dosage, no other toxic manifestations were observed.

SEMI-CONTROLLED STUDIES

In Figure 3 are shown the findings in an ambulatory outpatient, AKEL, under an insulin and mixed-diet regimen. This man was a cooperative patient, aged 65, who had severe diabetes of approximately one year's duration. Adequate control of the blood sugar level, glycosuria and ketonuria had not been achieved, despite a daily dosage of 50 units of insulin per

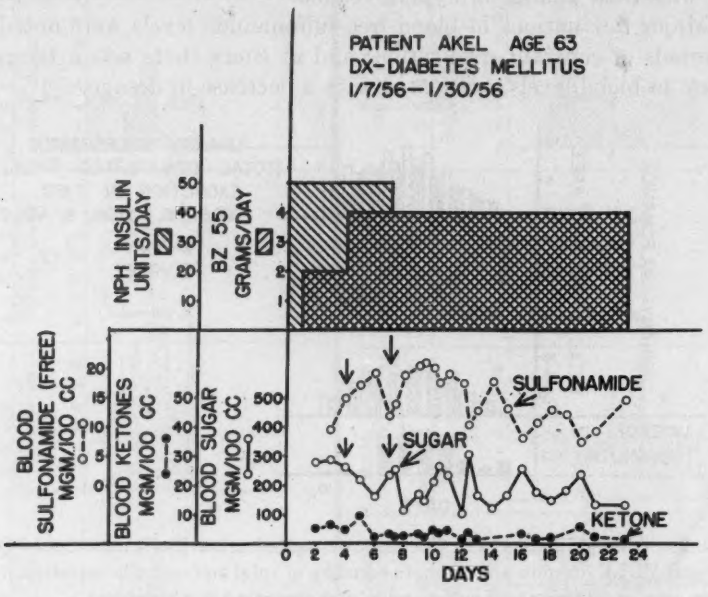

FIG. 3. Better diabetic control (decreased insulin requirement) in association with sulfonamide administration. Before BZ-55, the blood sugar level varied from 300 to 500 mg. per 100 cc. The apparent reciprocity between blood sulfonamide and sugar levels is of interest.

day. Fasting blood sugar levels ranged between 300 and 500 mg. per 100 cc. Qualitative tests all showed 3+ or 4+ sugar in the urine. Administration of BZ-55 was associated with a prompt decrease in the insulin requirement. The patient noted "blue urine tests" for the first time since the onset of his diabetes. Of particular interest is the apparently reciprocal relationship between the blood levels of free sulfonamide and of sugar. The fluctuations in free sulfonamide concentration were not related to the time of sulfonamide administration. Presumably they were attributable to erratic plasma protein binding of sulfonamide, to intra- and extra-cellular shifts in sulfonamide, to variation in the percentage of conjugation, and/or

to erratic sulfonamide excretion. Early in the course of BZ-55 administration, 24-hour thyroidal I^{131} uptake was found to be 2 per cent. At no time was there any clinical suggestion of thyroid dysfunction. This patient is now under study on the metabolic ward. His diet contains 101 grams of protein, 144 grams of carbohydrate, and 103 grams of fat. With this diet, and the administration of 25 units of NPH insulin and 2 grams of BZ-55 daily, the control of the diabetes is currently about optimal. "Orinase" will be substituted for BZ-55 in the immediate future. Specific metabolic findings in this patient will be reported in a later paper.

Patient RGAN, a 46-year-old man with severe diabetes of eighteen years' duration complicated by vascular disease and pulmonary tubercu-

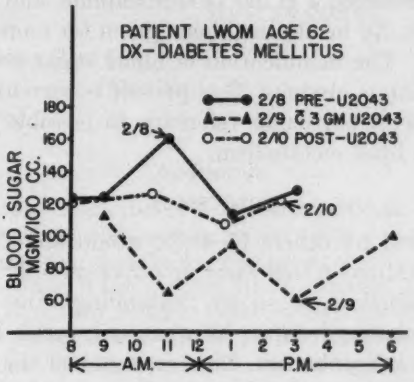

FIG. 4. Modification of blood sugar levels in response to 3 grams of U-2043 (Orinase).

losis, received large amounts of "Orinase," and later BZ-55, with no apparent effect on the insulin requirement. No crystalluria was noted during treatment with 6 grams of "Orinase" daily (in conjunction with potassium citrate and a large fluid intake). He will be re-studied at a later date under conditions of a chemically constant intake.

Patient RMOL, aged 31, a male "brittle diabetic" with progressive retinal and renal vascular disease, had an incomplete hypophysectomy on November 15, 1955. He has been maintained to date on varying dosages of sulfonamide. With a "clinically constant" diet, his insulin requirement was decreased by approximately half, but the fluctuations in blood sugar level continued to be of greater amplitude than one would wish. Further studies are in progress.

Patient PFOR, aged 40, with a strong family history of diabetes, was

found to have a distinctly diabetic glucose tolerance curve. Administration of 6 grams of sulfonamide for one day (diet remaining "clinically constant") was associated with the return of the glucose tolerance curve to nearly normal.

All of the foregoing patients were thin diabetics; and all except the last patient had severe diabetes in terms of insulin requirement and "brittleness."

In Figure 4 is shown as 12-hour study in a middle-aged moderately obese female diabetic, LWOM, maintained on a chemically constant diet administered at hourly intervals. For comparison, blood sugar levels at corresponding intervals are shown for 1) the day preceding medication 2) the day on which she received 3 grams of sulfonamide, and 3) the day following this medication. No insulin had been given for more than three weeks prior to this study. The modification of blood sugar levels during sulfonamide administration is obvious. This patient is currently under precisely controlled study, with particular reference to possible effects of the two sulfonamides upon lipid metabolism.

DISCUSSION

As has been noted by others (1–4), it would seem probable that the sulfonamide preparations which exert an effect directly or indirectly upon carbohydrate metabolism do so by "extending" the effects of insulin, rather than by replacing insulin. At what enzymatic level or levels this effect is exerted, is still unknown. The constancy of the blood and urinary ketones and of the nitrogen balances in the metabolic studies shown, despite major glycosuria and hyperglycemia in 1 patient so studied, suggest a primary effect upon lipid metabolism or over-all energy requirement. It seems improbable that the effects upon thyroid function bear any direct relationship to the effects upon carbohydrate metabolism.

The explanation for the totally different urinary sugar responses in 2 reasonably comparable subjects under almost identical metabolic programs, is unclear to us at present. The different patterns of excretion for free and for conjugated sulfonamides suggest different forms of hepatic metabolism of the sulfonamide which may be of importance in this regard.

The occurrence of neutropenia during *heavy* dosage with the sulfonamide indicates the need for caution in the use of these materials, particularly in view of the lag in complete elimination of sulfonamide from the organism, as indicated by the measurable amounts in the blood for some days after the last dose. Examination of the bone marrow in 1 patient by Dr. William Chew yielded no evidence of toxic effect. It seems not improbable that there is a chemical inhibition of the marrow, bearing a direct relationship to dosage.

Investigation in this laboratory is being concentrated upon the mechanism of action, and upon the use of these agents for possible stabilizing effects in "brittle" diabetics.

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Addendum

In an effort to evaluate mechanism of action, with particular reference to effects on fat metabolism, an additional study is being carried out in a 32-year-old male diabetic.

For a period of twenty-four days, he was maintained on a diet consisting almost entirely of fat—(carbohydrate, 0 Gm.; protein (from desiccated liver used as a dietary supplement), 6 Gm.; fat, initially butterfat (eighteen days) and subsequently cottonseed oil (six days), 223 Gm. per twenty-four hours). The diet was adequately fortified with essential vitamins, choline chloride and minerals, and was administered through a nasoduodenal tube, hourly, throughout the twenty-four hours (Fig. 5).

He received BZ-55 as shown. No insulin was administered at any time. (His insulin requirement on an average mixed diet had been 30 units daily, in the period immediately preceding hospital admission.) Throughout the course of study, the patient was in excellent health and spirits.

The following observations deserve comment:

1. The administered sulfonamide, in addition to eliminating hyperglycemia, appeared to have a favorable effect on hyperketonemia in this patient, despite a metabolic mixture (during the intake of cottonseed oil) in which the maximal available carbohydrate, derived from catabolized protein (urinary $N \times 6.25 \times 0.58$) and from the glycerol moiety of fat, was 37 grams. His minimal daily energy requirement was 1,800 calories. Therefore, he catabolized at least 184 grams of fatty acid daily. This represents a ratio of approximately 650 millimoles of fatty acid (as oleic acid) to 410 millimoles of triose (5). Previous studies carried out in this laboratory indicate that in the nondiabetic human subject on such a diet, clinical ketoacidosis can be prevented only by intensive corticoid administration (6). One of the effects of such treatment is major acceleration of neoglucogenesis from protein.

2. The rate of net disappearance of sulfonamide from the blood stream was rather slow after discontinuance of administration. The biologic half-time was 2.4 days, as compared to a biologic half-time of 1.4 days in Patients VGLE and JJON.

3. The total catabolism of protein approached the minimum.

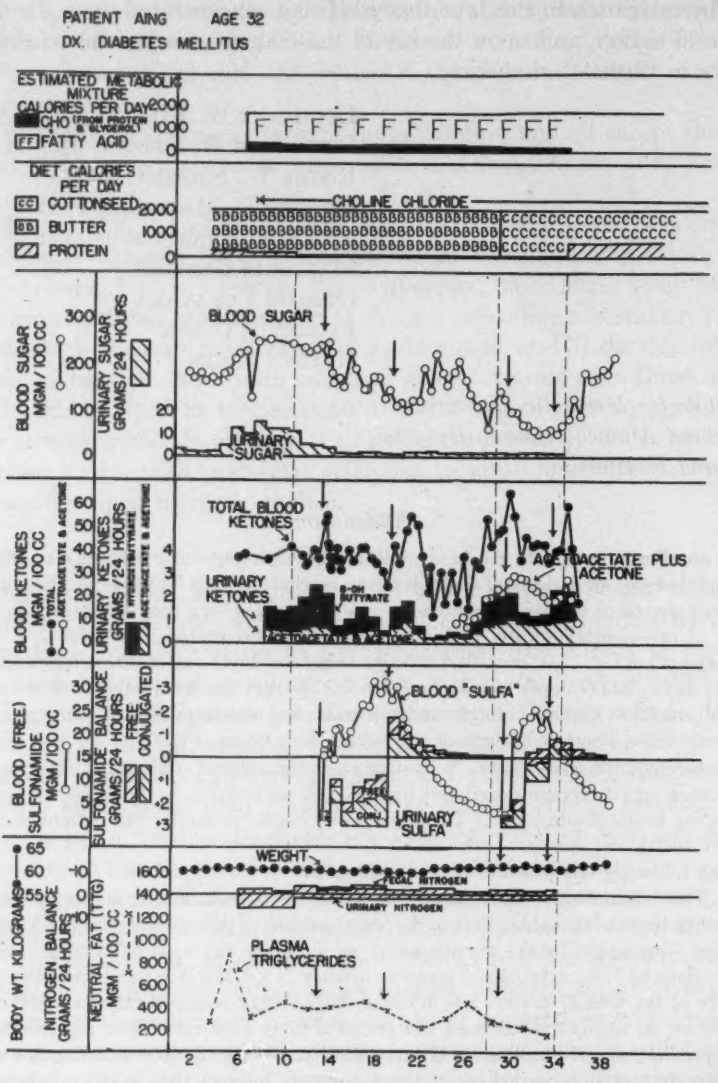

FIG. 5. Effects of sulfonamide upon energy metabolism in a patient maintained for twenty-four days on a diet containing essentially pure fat.

Nothing in the foregoing would appear to be incompatible with the concept that a primary effect of this sulfonamide is upon fat oxidation.

Studies with labeled and unlabeled acetate designed to clarify mechanisms, are in progress.

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CYTOLOGIC TESTS OF SEX IN CONGENITAL TESTICULAR HYPOPLASIA*

TO THE EDITOR:

The results of cytologic tests of sex in congenital errors of sex development, and theoretical considerations, indicated that patients with testes as the only type of gonadal tissue would be chromatin-negative[1] in all instances (1). This view had to be revised when a skin biopsy specimen from a patient with Klinefelter's syndrome, submitted to the Department of Microscopic Anatomy by Dr. Nicola D'Onghia of Milan, proved to be chromatin-positive. Following this lead, 2 cases of congenital testicular hypoplasia in adults, both with chromatin-positive nuclei in the epidermis, oral epithelium and testicular interstitial cells, have been studied. In one patient, biopsies of the cryptorchid testes showed abundant Leydig cells and numerous hyalinized seminiferous tubules. The histologic picture was similar to that in Klinefelter's syndrome. In the other patient, the testicu-

* Supported by grants from the Ontario Cancer Treatment and Research Foundation and the National Research Council of Canada.

[1] The more appropriate sex in congenital errors of sexual development is often at variance with the chromosomal sex, as inferred from cytologic tests. It has been suggested (2) that the terms chromatin-positive and chromatin-negative (referring to the sex chromatin) be used instead of female nuclei and male nuclei in clinical practice, to avoid placing an unnecessary psychologic burden on the patient.

lar biopsy showed poorly organized connective tissue, a few Leydig cells, and infrequent hyalinized seminiferous tubules that were so atrophic as to be barely recognizable.

These observations suggest that certain types of congenital testicular hypoplasia are the result of a genetic error in subjects with two X-chromosomes (XX or XXY?). The major defect is in the seminiferous tubules. The activity of the Leydig cells in the fetal gonads is apparently adequate for male differentiation of the reproductive tract (3). Cytologic tests of sex in such conditions as dystrophia myotonica, in which testicular hypoplasia is a conspicuous component of the syndrome (4), might yield new information concerning the genetic basis.

Although the nuclei of the epidermis and oral mucosa in the 2 patients cited could not be distinguished from those in normal females, study of blood films (5) yielded equivocal results. It was necessary to examine approximately 1,000 neutrophils to find 6 of them with a female morphology. Usually, the blood film findings are consistent with those obtained from skin biopsy or mucosal smear. We are unable to offer an explanation for the discrepancy in these particular patients.

A detailed report of our observations is in preparation.

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THYROIDAL FUNCTION IN MONGOLISM

TO THE EDITOR:

In their recent Letter to the Editor (*J. Clin. Endocrinol. & Metab.* **16**: 160 (Jan.) 1956) McGirr and Murray presented data which they in-

terpreted as showing that there is no evidence for an endocrine basis, either pituitary or thyroid, for mongolism. We should like to confirm their statement, because the opinion that mongolism is dependent upon some thyroid or pituitary deficiency is widespread, and reported in most treatises.

In a study of 14 young mongol patients we have found: a) a bone age corresponding to the real age, b) a normal basal metabolic rate, c) a mean plasma cholesterol level of 140 mg. per 100 ml., d) a mean serum protein-bound I^{127} level of 7.4 gamma per 100 ml., e) mean values for thyroidal I^{131} uptake—17.6 per cent after one hour, 43.1 per cent after twenty-four hours, 38.4 per cent after forty-eight hours, and f) a conversion ratio of 51 per cent. Three of these patients were given 200 microcuries of I^{131} and after forty-eight hours the butanolic extract of their plasma was analyzed by paper chromatography. Iodide, thyroxine and triiodothyronine were present, as in normal subjects. Adrenal function was also studied. The mean value for urinary 17-ketosteroids was 6.4 mg. per twenty-four hours and 11-hydroxycorticosteroids were normal. The Thorn test and the insulin tolerance test yielded normal results.

These findings (to be published in detail in "Folia Endocrinologica") confirm that there is no evidence for an endocrine basis for mongolism.

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G. M. Ferraris, M.D.
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Istituto per le malattie endocrine
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(Direttore, Prof. A. Costa),
Torino, Italy

EFFECT OF COBALT ON THYROIDAL UPTAKE OF I^{131}

To the Editor:

In 1955 Kriss, Carnes and Gross (1) reported the development of thyroid hyperplasia in 4 children and 1 adult receiving cobaltous chloride by mouth in dosages ranging from 2.8 to 3.9 mg./Kg./day. In 3 of the cases, thyroidal I^{131} uptake was studied and found to be profoundly depressed by cobalt. Jaimet and Thode (2) have thrown serious doubt upon the results of Kriss *et al.* Using cobaltous chloride in a dosage up to 6 mg./Kg./day for ten weeks, in the form of a cobalt-iron preparation, they found no significant change in thyroid function (thyroidal I^{131} uptake, conversion ratio, and salivary radioactivity) in 15 children; 2 other children showed a distinctly lowered thyroidal uptake after one course of cobalt, but no ef-

fect following a second course. According to Holly (3), the administration of cobaltous chloride (with or without iron, in dosages ranging from 60 to 100 mg. daily for ninety days) did not result in goiter formation in 78 pregnant women, nor in mice and rats given dosages up to 40 mg./Kg./day. However, no radioiodine studies were made. Scott and Reilly (4) found no difference in the thyroidal I^{131} uptake of rats treated with cobaltous chloride in a dosage of 60 mg./Kg./day, as compared with controls.

In view of the existing confusion regarding the effect of cobalt upon thyroidal I^{131} uptake, we wish to present briefly our findings in 12 adult subjects given cobaltous chloride for a short period of time (Fig. 1). These subjects were hospitalized for varying reasons, were in excellent shape, and were clinically euthyroid. None had anemia.

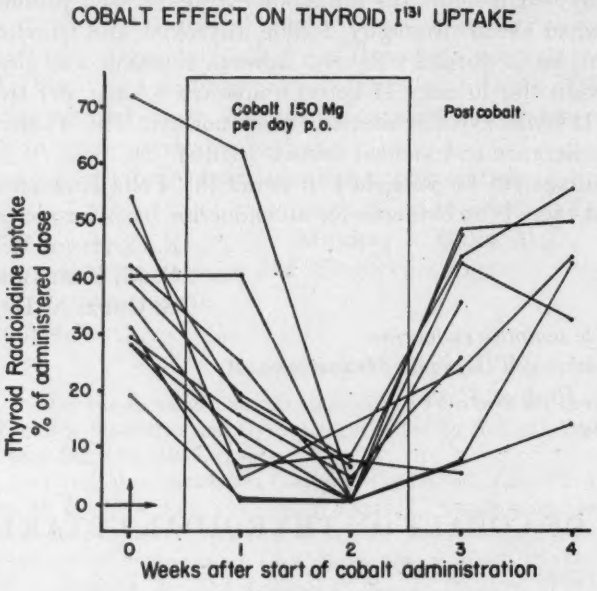

FIGURE 1

A dose of 25 microcuries of I^{131} was administered by mouth, and the 48-hour thyroidal I^{131} uptake was determined with a scintillation counter (Nuclear DS-1). Cobaltous chloride was then given daily for two weeks in a uniform dosage of 50 mg. three times daily by mouth. Thyroidal uptake measurements were repeated at weekly intervals, correcting the 48-hour value for residual thyroidal I^{131}. Except for 1 patient from an endemic goiter region, thyroidal uptakes were within the normal range before the administration of cobalt. Within one week after starting cobalt, the thy-

roidal I^{131} uptake values in most cases were greatly reduced and by the second week were near zero; they rose toward normal after cobalt was discontinued.

It seems clear from these results that cobaltous chloride given under these conditions interferes markedly with the uptake of I^{131} by the thyroid of adult patients. The mechanism of this interference, as well as its duration under prolonged administration of cobalt, is being investigated.

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Addendum

Since sending in this letter, we have had the opportunity, with the cooperation of Dr. E. Pimentel Malaussena, to treat 2 cases of hyperthyroidism with cobaltous chloride. The first patient was treated with 150 mg./day and later with 300 mg./day. Within two and a half months, her BMR fell from +60 to −1 per cent, thyroidal I^{131} uptake from 78 to 17 per cent, and conversion ratio from 76 to 5 per cent; salivary radioactivity rose from 5,289 to 24,247 counts/minute/3 cc. A thyroidectomy was performed without difficulty and the patient is well two weeks after operation. The second patient was treated with 300 mg. daily from the start. Within a month, her BMR fell from +22 to +5 per cent, thyroidal I^{131} uptake from 69 to 15 per cent, and conversion ratio from 93 to 6 per cent; salivary radioactivity rose from 363 to 56,388 counts/minute/3 cc. She is awaiting operation. Both patients showed marked clinical improvement, except for the body weight, which remained the same in spite of apparent good appetite.

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3. HOLLY, R. G.: Studies on iron and cobalt metabolism, *J.A.M.A.* **158:** 1349, 1955.
4. SCOTT, K. G., and REILLY, W. A.: Cobaltous chloride and iodine metabolism of normal tumor-bearing rats, *J.A.M.A.* **158:** 1355, 1955.

The Endocrine Society

THE 1956 ANNUAL MEETING

The Thirty-eighth Annual Meeting of The Endocrine Society will be held in the Palmer House, Chicago, Illinois, Thursday, Friday and Saturday, *June 7–8–9, 1956.*

HENRY H. TURNER, M.D.
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(For program, *see* April issue of the Journal)

AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers;

1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

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For further information concerning the program and registration, write to *Dr. J. B. Trunnell, M. D. Anderson Hospital, 6723 Bertner Avenue, Houston 25, Texas.* Registration is limited to 100; tuition fee is $100.00. Rooms will be reserved for the students and faculty at the Shamrock Hilton Hotel which is adjacent to the Texas Medical Center.

PUBLIC HEALTH SERVICE RESEARCH GRANTS

The Public Health Service has announced a new procedure to expedite the processing of research grant applications for those requests which do not exceed $2,000 plus indirect costs and which do not ask support for more than one year. Such applications will be accepted and processed on receipt and are not therefore subject to the usual deadlines for submission prior to review.

Council recommendations can be expected on these applications within 1–4 months from the time of submission. These procedures do not apply for requests for supplements to existing grants.

Address all applications as well as requests for forms or additional information to the Division of Research Grants, National Institutes of Health, Bethesda 14, Maryland.

DICKINSON RESEARCH MEMORIAL GRANTS

The Dickinson Research Memorial, research arm of the Planned Parenthood Federation of America, Inc., is interested in supporting basic research in the physiology of reproduction, with special emphasis on factors influencing fertility. For further information address the Dickinson Research Memorial, 501 Madison Avenue, New York 22, N. Y.

CORRECTION

In the article entitled "The Effect of Iodide on the Release of Thyroid Hormone in Hyperthyroidism" by R. E. Goldsmith and M. L. Eisele, *J. Clin. Endocrinol. & Metab.* **16**: 130–137 (Jan.) 1956, the authors wish to make the following corrections: on page 134, in the legend to Figure 13, "trichloracetic-acid precipitation" should read "Somogyi precipitation"; and on page 135, line 12, "trichloracetic-acid precipitable fraction" should read "Somogyi-reagent precipitable fraction."

BOOK REGISTER

A Pictorial History of Medicine. By OTTO L. BETTMANN, Ph.D., Founder and Director of The Bettmann Archive, New York City, with a Foreword by PHILIP S. HENCH, M.D., Mayo Clinic, Rochester, Minn. A brief nontechnical survey of the healing arts from Aesculapius to Ehrlich, retelling with the aid of select illustrations the lives and deeds of great physicians—a graphic record of the untiring efforts of the medical profession to cure the sick, offer solace, allay pain and secure a better life for all mankind. Its chapters are subdivided into compact thematic units of text and illustration. Each unit covers a particular subject, but the units keep their logical places in the general text; 334 pages; profusely illustrated; 1956. Charles C Thomas, Publisher, Springfield, Illinois.

Endocrinologia del Crecimiento. Ciclo de Conferencias Pronunciadas en la Facultad de Ciencias Medicas de Buenos Aires. DR. RODOLFO Q. PASQUALINI, Professor Titular de Clinica Médica, Director del Instituto Nacional de Endocrinologia; 297 pages; illustrated; 1955. La Prenza Médica Argentina, Buenos Aires.

Essays in Biochemistry. Edited by SAMUEL GRAFF. Essays written in honor of *Hans Thacher Clarke,* on the occasion of his retirement as Professor and Chairman of the Department of Biochemistry, College of Physicians and Surgeons, Columbia University. The essays survey the present status of biochemistry, discuss some major research problems, and speculate on future trends; 355 pages; illustrated; 1956. John Wiley & Sons, Inc., 440 Fourth Avenue, New York, N. Y. Price $6.50.

Hypothalamic-Hypophysial Interrelationships. Edited by WILLIAM S. FIELDS, Professor of Neurology, ROGER GUILLEMIN, Assistant Professor of Physiology, and CHARLES A. CARTON, Assistant Professor of Neurological Surgery, Baylor University College of Medicine. A Symposium, Third Annual Scientific Meeting of the Houston Neurological Society, Texas Medical Center, Houston, Texas—of special interest to endocrinologists, internists, neurologists, neurophysiologists and psychiatrists, as well as to basic science research workers. An up-to-date presentation of the principal problems relating to the mechanisms which control the release of the various hormones of the hypophysis under homeostatic physiological conditions and in stress. New techniques are introduced. Various concepts are presented regarding the anatomic and physiologic relationships between the hypothalamus and the hypophysis, with special attention to neural connections, blood vessels, and the mechanism and role of neurosecretion; 166 pages; illustrated; 1956. Charles C Thomas, Publisher, Springfield, Illinois. Price $4.75.

1956 Medical Progress. A Review of Medical Advances During 1955. Edited by MORRIS FISHBEIN, M.D., Professor of Medicine, University of Illinois College of Medicine, Chicago, Illinois; 21 chapters on various aspects of medicine, by authorities in the field; the subjects cover medicine, allergy, rheumatic diseases, diabetes, gynecology, gastroenterology, laboratory procedures, general surgery, ophthalmology, poliomyelitis vaccine, neurology, physical medicine and rehabilitation, dermatology, nutrition, cardiovascular disease, psychiatry, neuropsychiatry, new drugs, endocrinology, ear, nose and throat, and orthopedic surgery; 399 pages; 1956. McGraw-Hill Book Company, Inc., 330 West 42nd St., New York, N. Y. Price $5.50.

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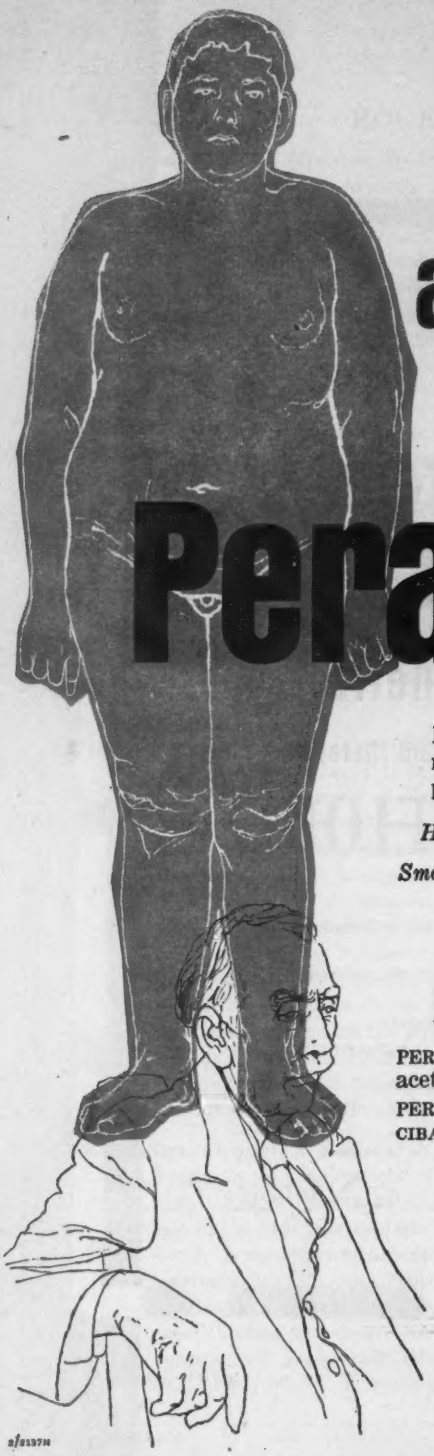

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References to books should be listed as follows: OSLER, W.: Modern Medicine, ed. 3. Philadelphia, Lea & Febiger, 1927, vol. 5, p. 66.

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By

HUGH JOLLY, M.A., M.D., Camb., M.R.C.P.
Consultant Paediatrician, Plymouth Clinical Area, London, England

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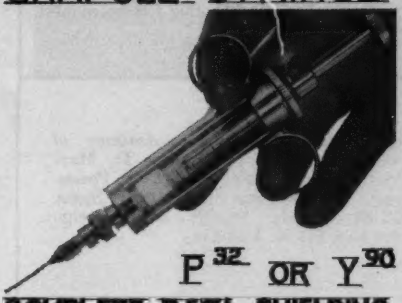

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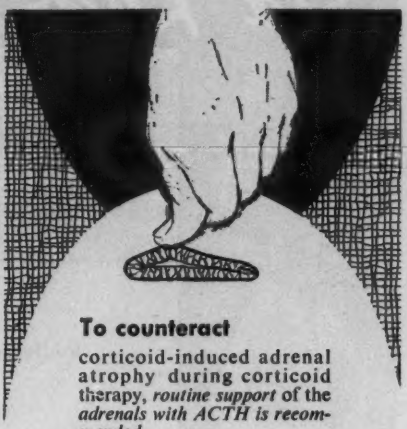

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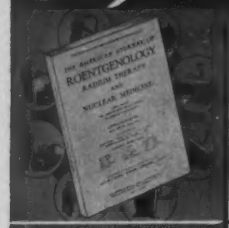

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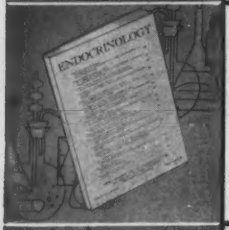

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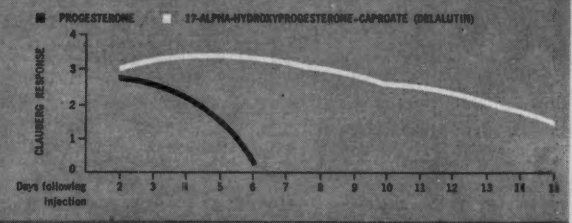

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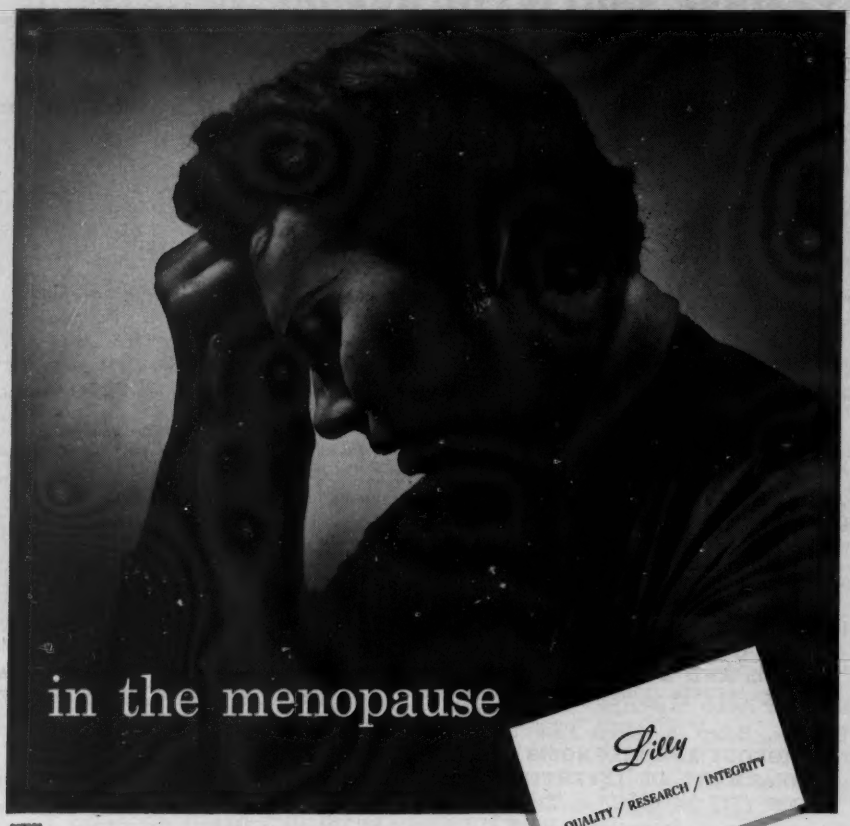

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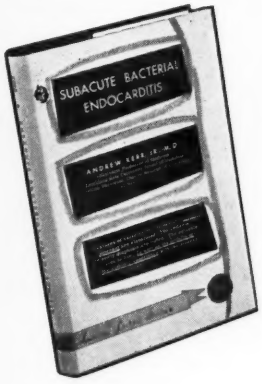

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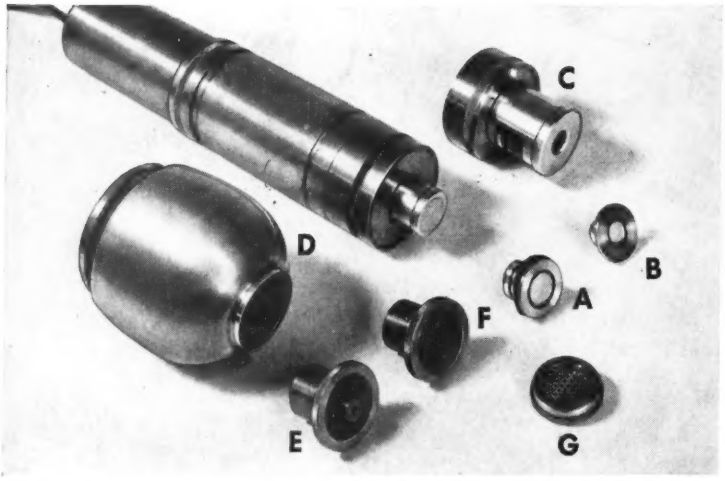

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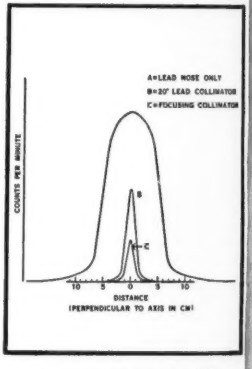

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